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(54) Title: GLYCOSYLATION MODIFIED IL-20

(57) Abstract: This invention relates to glycosylation-modified IL-20 polypeptides which preferentially signal through one of the multiple IL-20 receptor complexes and maintain at least one activity associated with wild type IL-20 while decreasing or eliminating at least one other activity associated with wild type IL-20. The N-linked consensus glycosylation sites are engineered into IL-20 at one or more sites which correspond to an N-linked consensus glycosylation site that exists in wild type IL-19 or IL-24. The invention also relates to polynucleotides encoding the polypeptides of the invention, vectors and host cells comprising a polynucleotide of the invention, and therapeutic methods for using the polypeptides and polynucleotides of the invention.

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GLYCOSYLATION MODIFIED IL-20

Field of the Invention

5 The present invention relates to novel, glycosylation-modified IL-20 polypeptides that preferentially signal through one of the IL-20 receptor complexes and polynucleotides that identify and encode the polypeptides. Also provided are vector, host cells and recombinant methods for producing the same. The invention further provides therapeutic methods for using the polypeptides and polynucleotides of the invention.

10

Background of the Invention

Interleukin 20 (IL-20) is a member of the interleukin-10 (IL-10) protein family. Additional members of this protein family include, but are not limited to, interleukin 19 (IL-19) and interleukin 24 (IL-24). These proteins share limited primary sequence
15 identity, some structural homology and receptor subunits (for review see Kotenko, S., Cytokine & Growth Factor Reviews 13:223, 2002).

The IL-20 polynucleotide and polypeptide sequences are described in International Patent Publication Nos. WO 99/27103 and WO 00/12708. The IL-19 polynucleotide and polypeptide sequence are described in U.S. Patent 5,985,614 and the IL-24 polynucleotide
20 and polypeptide sequence are described in International Patent Publication No. WO 95/11986 and Jiang, H. & Fisher, P., Mol. Cell. Differ. 1:285, 1993. Functions of the members of the IL-10 protein family are only recently being revealed and much remains to be learned. IL-10 plays major roles in inflammatory and immune responses (Moore, K. et al. Ann. Rev. Immunol. 19:683, 2001). Some of the other members of the IL-10
25 protein family have been shown to be involved in the regulation of inflammatory responses in various tissues. The function of IL-19 is unknown although its expression closely follows (temporally) the expression of IL-10 which may suggest a role for IL-19 as a feedback inhibitor of proinflammatory cytokine production, similar to IL-10, or it may limit the anti-inflammatory action of IL-10 (Kotenko, S., Cytokine & Growth Factor
30 Reviews 13:223, 2002). IL-24 has been shown to be associated with chromatin in cells undergoing mitosis and it has also been linked to the promotion of apoptosis in cancer cells (Su, Z., et al., Proc. Natl. Acad. Sci. 95:14400, 1998, U.S. Patents 6,355,622 and

5,710,137). IL-20 has been linked to skin development and stimulation of platelet proliferation (International Patent Publication No. WO 99/27103), as well as activity resulting in an increased number of CFU-GEMM cells which are hematopoietic progenitor cells of red blood cells, platelets, granulocytes and monocytes (U.S. Patent Applications with serial numbers 60/272,242 filed February 28, 2001; 60/332,740 filed November 19, 2001 and 60/353,789 filed February 1, 2002) and anti-obesity activity (International Patent Application No. PCT/US02/00498).

Cytokines such as IL-20, IL-19 and IL-24, are secreted proteins that exert their actions by binding to specific cell-surface receptors that leads to signaling, *i.e.*, activation of cytokine-specific signal transduction pathways. A cytokine that signals through multiple, different receptor complexes may thereby activate multiple, different signal transduction pathways. All IL-10-related cytokines signal through receptors belonging to the class II cytokine receptor family (Bazan, J., Proc. Natl. Acad. Sci., 87:6934, 1990). These ligands signal through receptor complexes composed of two distinct receptor chains, both of which must be simultaneously present in the same cell, leading primarily to the activation of the Jak-Stat signal transduction pathway. The class II cytokine receptor subunits can be referred to as R1 and R2. In the established paradigm for this receptor family, the R1 subunit binds the ligand with high affinity, has a long intracellular domain which is associated with Jak1 tyrosine kinase, becomes phosphorylated at tyrosine residues after ligand binding and then acts to recruit various signal transducing proteins to the receptor complex. R1 defines the specificity of the signaling. The R2 subunit acts to initiate signal transduction by bringing an additional tyrosine kinase to the receptor complex. IL-20 does not follow the established receptor-binding paradigm and requires both receptor subunits for high affinity binding and does not detectably bind to either subunit expressed alone (Blumber, H. *et al.* Cell 104:9, 2001).

Despite their variety of demonstrated functions, IL-20, IL-19 and IL-24 share some receptor subunits (Dumoutier, L, *et al.*, J. Immunology 167:3545, 2001; Wang, M. *et al.*, J. Biol. Chem. 277:7341, 2002). The IL-19 receptor complex consists of IL20R1 and IL20R2 subunits (U.S. Patent No. 5,945,511 and International Patent Application No. PCT/US99/03735). Both IL-20 and IL-24 signal through two receptor complexes one of which is identical to the IL-19 receptor complex, the other consists of IL22R1 and IL20R2 subunits. Thus IL20R2 is common to both receptor complexes used by IL-20 and IL-24.

Some studies have reported the presence of these receptor subunits in particular tissues, but none have shown which of the receptor subunits, if any, are expressed in sources that contain hematopoietic stem cells such as the bone marrow or the thyroid and would therefore be available for IL-20 to activate CFU-GEMM proliferation (summarized in
5 Kotenko, S., Cytokine & Growth Factor Reviews 13:223, 2002). The diverse biological activities of IL-20, that likely result from signaling through a particular receptor for a particular activity, have led to a need for glycosylation-modified IL-20 polypeptides, and polynucleotides that encode them, which preferentially signal through one of the IL-20 receptor complexes and thereby preserve a particular therapeutic utility while decreasing
10 or eliminating another utility.

Summary of the Invention

The present invention addresses the need for modified forms of IL-20 polypeptides capable of preferentially signaling through one IL-20 receptor complex, and related
15 compositions and methods.

The present invention embodies a glycosylation-modified IL-20 polypeptide that preferentially signals through one of the IL-20 receptor complexes, preferably at a level at least 1.3, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 or 9.0 times the level it signals through a different IL-20 receptor complex. Preferably the preferred IL-20 receptor complex
20 comprises IL20R1 and IL20R2 subunits.

The invention embodies multiple forms of glycosylation-modified IL-20 polypeptides. For example, it is contemplated that the glycosylation-modified IL-20 polypeptides of the invention encompass natural allelic variants. The invention further contemplates a glycosylation-modified IL-20 polypeptide that preferentially signals
25 through one of the IL-20 receptor complexes and comprises an amino acid sequence at least about 95%, even more preferably at least 96%, 97%, or 98% and most preferably at least 99% identical or homologous (*i.e.*, amino acid sequence identity) to the sequence of amino acids 25 through 125, amino acids 1 through 125, amino acids 25 through the C-terminus, or amino acids 1 through the C-terminus, of SEQ ID NO: 5, 6, 7, 8, 14, 15, 16,
30 17, 18, 19, 20 or 21 in which the N-linked glycosylation consensus sequences [NX(T/S)] are such that N is an asparagine amino acid, X is any amino acid, preferably not a proline, and T/S is either a threonine or a serine (see SEQ ID NOs: 14-17). It is further

contemplated that the N-linked glycosylation sequences of the glycosylation-modified IL-20 polypeptides of the invention may be shifted from their existing position, as shown in SEQ ID NOs: 5-9 and 14-21, in either direction, by 1, 2 or 3 amino acids.

5 The polypeptides of the present invention may have the IL-20 signal sequence replaced with the signal sequence of a different protein.

The invention embodies glycosylation-modified IL-20 polypeptides that have one or more of the glycosylation modifications illustrated in SEQ ID NOs: 14-17. For example, a glycosylation-modified IL-20 polypeptide may have two N-linked glycosylation sites, one at amino acids 61-63 as shown in Gly4 (SEQ ID NO: 8 or 17) and
10 one at amino acids 140-142 as shown in Gly1 (SEQ ID NO: 5 or 14; see SEQ ID NOs: 18-21). It is noted that the N-linked glycosylation site for all polypeptides of the invention need not be that sequence specifically illustrated in SEQ ID NOs. 5-8, they must only satisfy the consensus sequence required for an N-linked glycosylation site, i.e., NXT/S as described above and exist at one or more of the following positions within IL-
15 20: amino acids 61-63 (site 1), amino acids 97-99 (site 2) and 140-142 (site 3) or shifted to either side of these positions by 1, 2 or 3 amino acids (see, e.g., SEQ ID NOs: 18-21).

The invention embodies a fusion polypeptide comprising a first portion and a second portion joined by a peptide bond. The first portion of the fusion polypeptide comprises amino acids 25 through 125, amino acids 1 through 125, amino acids 25
20 through the C-terminus, or amino acids 1 through the C-terminus, of a glycosylation-modified IL-20 polypeptide or variant thereof, with or without the signal sequence. The second portion of the fusion polypeptide consists of another polypeptide including, but not limited to, an affinity tag. Within one exemplary embodiment, the affinity tag is an immunoglobulin Fc polypeptide. Within another exemplary embodiment the affinity tag is
25 FLAG and/or His6. For fusion polypeptides in which the first portion does not provide a signal sequence; the signal sequence is provided by the second portion of the fusion polypeptide. The first portion of the fusion polypeptide may be operably linked to the amino terminal or carboxy terminal end of the second portion.

The present invention further embodies glycosylation-modified IL-20 polypeptides
30 comprising, or alternatively, consisting of, a polypeptide selected from the group consisting of about amino acids 25 through 125, amino acids 1 through 125, amino acids

25 through the C-terminus, and amino acids 1 through the C-terminus of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21.

The present invention embodies a nucleic acid molecule comprising a polynucleotide encoding a glycosylation-modified human IL-20 polypeptide that preferentially signals through one IL-20 receptor complex, preferably at a level at least 1.3, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, or 9.0 times the level it signals through a different IL-20 receptor complex.

The invention further embodies a nucleic acid molecule comprising a polynucleotide encoding a glycosylation-modified human IL-20 polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 or 21.

The present invention further embodies a nucleic acid molecule comprising a polynucleotide encoding a glycosylation-modified human IL-20 having an amino acid sequence at least about 95%, even more preferably at least 96%, 97%, or 98% and most preferably at least 99% identical (*i.e.*, amino acid sequence identity) to that shown in SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20, or 21, in which the N-linked glycosylation consensus sequences [NX(T/S)] are such that the N is an asparagine amino acid, the X is any amino acid, preferably not a proline, and the T/S is either a threonine or a serine. Furthermore, the glycosylation-modified human IL-20 molecules encoded by the nucleic acid molecules of the invention may have a NX(T/S) sequence shifted in either direction by 1, 2 or 3 amino acids from where it exists in SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20, or 21 and/or may optionally have the IL-20 signal sequence replaced with the signal sequence of a different protein.

The invention further embodies a nucleic acid molecule comprising a polynucleotide with the sequence shown in SEQ ID NOs: 10, 11, 12, or 13, with or without the sequence encoding the first 24 amino acids (signal sequence). Those nucleic acid molecules not encoding the IL-20 signal sequence are operably linked to a nucleic acid molecule encoding a different signal sequence thereby enabling secretion of the polypeptide encoded by the nucleic acid molecule upon expression.

The present invention also embodies a recombinant vector, preferably an expression vector, that comprises a nucleic acid molecule of the present invention and a host cell, preferably a mammalian cell, comprising said recombinant vector. The

invention also provides a vector, preferably an expression vector, encoding a fusion polypeptide of the invention and a host cell, preferably a mammalian cell, transfected with such a vector to produce a fusion polypeptide of the invention. The invention also embodies methods of making such vectors and host cells of the invention and methods for
5 using them for production glycosylation-modified IL-20 polypeptides.

In other embodiments, the invention provides a method of modulating the physiology or development of a cell *in vivo* or *in situ* comprising introducing into such cell, or the environment of such cell, a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide of the invention.

10 The present invention embodies use of a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide of the invention in treating hematopoietic disorders, cancer, cardiovascular disorders, skin disorders such as psoriasis, inflammation and/or immune system disorders and pharmaceutical compositions comprising said polypeptide.

15 The invention further embodies a pharmaceutical composition comprising, alternatively consisting of or consisting essentially of, a hematopoietic progenitor cell-stimulating amount, or alternatively a CFU-GEMM cell-stimulating amount of at least one glycosylation-modified IL-20 polypeptide and/or variant thereof and a pharmaceutically acceptable carrier, diluent or excipient.

20

Brief Description of the Figures

Fig. 1 provides an alignment of the amino acid sequences of wild-type IL-20, IL-19, IL-24 and the glycosylation-modified IL-20 named Gly1, Gly2, Gly3 and Gly4 with
25 the N-linked glycosylation sites underlined.

Detailed Description

Definitions

The term "amino acid" is used herein in its broadest sense, and includes naturally
30 occurring amino acids as well as non-naturally occurring amino acids, including amino acid analogs and derivatives. The latter includes molecules containing an amino acid moiety. Reference herein to an amino acid includes naturally occurring proteogenic L-

amino acids; D-amino acids; chemically modified amino acids, such as amino acid analogs and derivatives; naturally occurring non-proteogenic amino acids such as norleucine, β -alanine, ornithine, etc.; and chemically synthesized compounds having properties known in the art to be characteristic of amino acids.

5 The term "glycosylation-modified IL-20 polypeptide" as used herein refers to an IL-20 polypeptide (SEQ ID NO: 1) with or without about the first (amino terminal) 24 amino acid signal sequence, said polypeptide modified to contain one or more N-linked glycosylation sites at amino acid positions 61-63, 97-99 and/or 140-142 or 1, 2, or 3 amino acids in either direction of these positions. Additionally, the term "glycosylation-
10 modified IL-20 polypeptide" includes glycosylation-modified IL-20 variants as described herein. The sequence of exemplary glycosylation-modified IL-20 polypeptides is shown in SEQ ID NOs: 5-8, 14-21.

 The term "N-linked glycosylation site" as used herein, refers to a 3-amino acid sequence (or the nucleotides encoding said sequence) of NX(T/S) in which the N is an
15 asparagine amino acid, the X is any amino acid, and the T/S is either a threonine or a serine amino acid.

 The term "glycosylation-modified IL-20 polynucleotide" as used herein refers to a polynucleotide that encodes a glycosylation-modified IL-20 polypeptide or a glycosylation-modified IL-20 variant polypeptide. The term further refers to a
20 glycosylation-modified IL-20 variant polynucleotide as described herein.

 The term "glycosylation-modified IL-20 variant" as used herein refers to a glycosylation-modified IL-20 polynucleotide or IL-20 polypeptide, e.g., as shown in SEQ ID NOs: 10-13 (polynucleotides), or 5-8 or 14-21 (polypeptides), that further comprises at least one of the various types of modifications contemplated herein. The methods of the
25 present invention contemplate alterations in a glycosylation-modified IL-20 polypeptides that may include one or more amino acid insertions, deletions, substitutions, truncations, fusions, shuffling of subunit sequences, and the like, either from natural mutations (e.g., allelic variants) or human manipulation, provided that the sequences produced by such modifications have substantially the same activity(ies) as the glycosylation-modified IL-
30 20 polypeptides (i.e., the parent molecule) without such additional modification(s). It is contemplated that such modification should result in a polypeptide no less than 95% homologous to the original glycosylation-modified IL-20 polypeptide sequence.

Furthermore, glycosylation-modified IL-20 variant, as applied to a polypeptide, is intended to refer to a "functional" or "active" glycosylation-modified IL-20 polypeptide, having at least about 95% amino acid sequence identity with a glycosylation-modified IL-20 polypeptide having the deduced amino acid sequences as shown in SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 or 21, with or without the signal sequence, and in which the N-linked glycosylation site may be shifted 1, 2, or 3 amino acids in either direction. Such glycosylation-modified IL-20 polypeptide variants include, for instance, glycosylation-modified IL-20 polypeptides wherein one or more amino acid residues are added, substituted or deleted, at the N- or C-terminus or within the sequence of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 or 21. Ordinarily, a glycosylation-modified IL-20 polypeptide variant will have at least about 95%, 96% or 97% amino acid sequence identity, preferably at least about 98% amino acid sequence identity, yet more preferably at least about 99% amino acid sequence identity with the amino acid sequence as shown in SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 or 21, with or without the signal peptide and in which the amino acids of an N-linked glycosylation site may be shifted 1, 2, or 3 amino acids in either direction. Variants are contemplated to include natural allelic variants of IL-20, i.e., encoded by the various alleles present in a population of mammals, preferably humans. Variants are also contemplated to include polypeptides comprising a fusion polypeptide in which the glycosylation-modified IL-20, or variant thereof, is operably linked to another polypeptide, e.g., an polypeptide encoding an affinity tag. In such a fusion polypeptide the second portion (i.e., that portion that is not a glycosylation-modified IL-20 polypeptide or variant thereof) is contemplated to not contribute to the percent identity with the target polypeptide.

The term "glycosylation-modified IL-20 variant polynucleotide" refers to a polynucleotide that encodes a glycosylation-modified IL-20 variant polypeptide as described above or a polynucleotide that has at least about 95%, 96%, 97% polynucleotide sequence identity, preferably at least about 98% polynucleotide sequence identity, yet more preferably at least about 99% polynucleotide sequence identity with the polynucleotide sequence shown in SEQ ID NOs: 10, 11, 12, or 13, with or without the sequence encoding the first 24 amino acids (the signal sequence) and in which the nucleotides encoding an N-linked glycosylation site may be shifted 3, 6, or 9 nucleotides (the equivalent of 1, 2, or 3 amino acids) in either direction.

As used herein, the terms "complementary" or "complementarity" are used in reference to nucleic acids (i.e., a sequence of nucleotides) related by the well-known base-pairing rules that A pairs with T and C pairs with G. For example, the sequence 5'-A-G-T-3', is complementary to the sequence 3'-T-C-A-5'. Complementarity between two single-stranded molecules can be "partial," in which only some of the nucleic acid bases are matched according to the base pairing rules. On the other hand, there may be "complete" or "total" complementarity between the nucleic acid strands when all of the bases are matched according to base pairing rules.

It will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequence of IL-20 may exist within a population (e.g., the human population). Such genetic polymorphism in the IL-20 gene may exist among individuals within a population due to natural allelic variation. An "allelic variant" is an alternate form of a polynucleotide sequence that may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide. Any and all such nucleotide variations and resulting amino acid polymorphisms in IL-20 that are the result of natural allelic variation(s) and that do not substantially alter the functional activity of the IL-20 polypeptide are intended to be within the scope of the invention when they exist in a glycosylation-modified IL-20 polynucleotide and the resulting polypeptide.

A polynucleotide of the present invention can be composed of any RNA or DNA, which may be unmodified or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded regions. A polynucleotide may contain one or more modified nucleotides. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of such modifications can be made; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

"Percent (%) amino acid sequence identity" with respect to the glycosylation-modified IL-20 amino acid sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence (e.g., glycosylation-modified IL-20) that are

identical with the amino acid residues in a reference polypeptide (e.g., wild type IL-20) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN, ALIGN-2, Megalign (DNASTAR) or BLAST (e.g., Blast, Blast-2, WU-Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, the percent identity values used herein can be generated using WU-BLAST-2 (Altschul *et al.*, Methods in Enzymology 266:460, 1996). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, (*i.e.*, the adjustable parameters) are set with the following values: overlap span = 1; overlap fraction = 0.125; word threshold (T) = 11; and scoring matrix = BLOSUM 62. For purposes herein, a percent amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the polypeptide of interest and the comparison amino acid sequence of interest (*i.e.*, the sequence against which the polypeptide of interest is being compared) as determined by WU-BLAST-2, by (b) the total number of amino acid residues of the polypeptide of interest, respectively.

"Percent (%) nucleic acid sequence identity" with respect to the polynucleotide sequences identified herein is defined as the percentage of nucleotides in a candidate sequence (e.g., glycosylation-modified IL-20) that are identical with the nucleotides in the reference sequence (e.g., wild type IL-20) after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN, Align-2, Megalign (DNASTAR), or BLAST (e.g., Blast, Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent nucleic acid identity values can be generated using the WU-BLAST-2 (BlastN module) program (Altschul *et al.*, Methods in Enzymology

266:460, 1996). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set default values (*i.e.*, the adjustable parameters), are set with the following values: overlap span = 1; overlap fraction = 0.125; word threshold (T) = 11; and scoring matrix = BLOSUM62. For purposes herein, a percent nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the polypeptide-encoding nucleic acid molecule of interest and the comparison nucleic acid molecule of interest (*i.e.*, the sequence against which the polypeptide-encoding nucleic acid molecule of interest is being compared) as determined by WU-BLAST-2, by (b) the total number of nucleotides of the polypeptide-encoding nucleic acid molecule of interest.

The term "mature protein" or "mature polypeptide" as used herein refers to the form(s) of the protein as would be produced by expression in a mammalian cell. For example, it is generally hypothesized that once export of a growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal peptide (SP) sequence which is cleaved from the complete polypeptide to produce a "mature" form of the protein. Oftentimes, cleavage of a secreted protein is not uniform and may result in more than one species of mature protein. The cleavage site of a secreted protein is determined by the primary amino acid sequence of the complete protein and generally cannot be predicted with complete accuracy. Methods for predicting whether a protein has an SP sequence, as well as the cleavage point for that sequence, are known in the art. A cleavage point may exist within the N-terminal domain between amino acid 10 and amino acid 35. More specifically the cleavage point is likely to exist after amino acid 15 but before amino acid 31. Cleavage sites sometimes vary from organism to organism and may even vary from molecule to molecule within a cell and cannot be predicted with absolute certainty. Optimally, cleavage sites for a secreted protein are determined experimentally by amino-terminal sequencing of the one or more species of mature proteins found within a purified preparation of the protein.

IL-20 (and glycosylation-modified forms of IL-20) has a signal sequence extending from amino acid residue 1, a methionine, and including amino acid residue 24, a glycine, of SEQ ID NO: 1. Thus, the mature IL-20 or mature form of any glycosylation-modified IL-20 extends from about amino acid residue 25, a leucine; to and including the C-terminal amino acid residue at position 176, a glutamic acid.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in the correct reading frame. However, enhancers do not have to be in the same reading frame.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous DNA into host cells. A vector comprises a nucleotide sequence that may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

The terms "treating", "treatment" and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventive therapy. An example of "preventive therapy" is the prevention or lessened targeted pathological condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

A "therapeutically-effective amount" is the minimal amount of active agent (*e.g.*, a glycosylation-modified IL-20 polypeptide) necessary to impart therapeutic benefit to a mammal. For example, a "therapeutically-effective amount" to a mammal is such an amount that reduces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression, physiological conditions associated with or resistance to succumbing to the aforescribed disorder.

As used herein, the term "inhibit" refers to a decrease, whether partial or whole, in function or activity. For example, inhibition of signaling activity refers to any decrease in signaling activity that is being measured (directly or indirectly), including complete elimination of said activity.

"Active" or "activity" or "functional" in the context of a glycosylation-modified IL-20 polypeptide, or variant thereof, refers to retention of a biological function of the wild type IL-20 polypeptide including *e.g.*, the ability to induce production of an antibody

against an antigenic epitope possessed by the IL-20 polypeptide at levels near that of the wild type IL-20 polypeptide, or preferably the ability to signal through one of the IL-20 receptors. "Activity" or "function" also refers to a biological function (either inhibitory or stimulatory) caused by a reference polypeptide (e.g., wild type IL-20). Exemplary biological activities include, but are not limited to, the ability of such molecules to inhibit inflammation, to increase the presence of CFU-GEMM cells, to treat the following: psoriasis or other skin disorder, an immune system disorder, inflammation, obesity, a hematological disorder, a cancer and/or a cardiovascular disorder.

Glycosylation-modified IL-20 polypeptides of the invention are typically administered to a subject in "substantially pure" form. The term "substantially pure" as used herein refers to a glycosylation-modified IL-20 polypeptide that is substantially free of other proteins, lipids, carbohydrates, or other materials with which it is naturally associated. One skilled in the art can purify a glycosylation-modified IL-20 polypeptide using standard techniques for protein purification.

Cells which are targeted by the methods of the present invention, such as, e.g., stem cells, hematopoietic stem cells or keratinocytes include isolated cells maintained in culture as well as cells within their natural context *in vivo*.

Overview

The present invention is based in part upon the discovery and synthesis of glycosylation-modified IL-20 polypeptides that preferentially signal through one of the multiple IL-20 receptor complexes, preferably at a level at least 1.3, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 or 9.0 times the level it signals through a different IL-20 receptor complex. The glycosylation-modified IL-20 polypeptides may have altered receptor binding, not because of the particular glycosylation group *per se*, but rather, because there is a bulky group now attached to the IL-20 molecule at a position resulting in receptor-binding inhibition. Example 5 demonstrates glycosylation-modified IL-20 polypeptides that preferentially signal through the receptor complex comprising IL20R1 and IL20R2 subunits. The present invention is further based upon the discovery of glycosylation-modified IL-20 polypeptide and their use in treating, preventing, and diagnosing hematopoietic disorders, obesity, cancer, cardiovascular disorders, skin disorders such as psoriasis, and/or immune system disorders such as autoimmune diseases and inflammatory disorders.

Glycosylation Modified IL-20

A type of covalent modification of the polypeptides included within the scope of this invention comprises altering the glycosylation pattern of the wild type IL-20 polypeptide.

- 5 "Altering the native glycosylation pattern" is intended for purposes herein to mean adding one or more N-linked glycosylation sites that are not present in the native sequences of IL-20. These sites are preferably added at a location equivalent to where a glycosylation site exists within IL-19 and/or IL-24 or within 1, 2 or 3 amino acids of said site.

- 10 Addition of glycosylation sites to polypeptides may be accomplished by altering the amino acid sequence thereof. The amino acid sequences may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids (NX(T/S)). Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such
- 15 methods are described in the art, e.g., in International Patent Publication No. WO 87/05330 and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

- The polypeptides of the present invention are glycosylation modified IL-20. These molecules have one or more N-linked glycosylation sites at amino acids 61-63, 97-99 and/or 140-142 (numbering based on full-length IL-20 protein sequence as shown in SEQ ID
- 20 NO: 1). The location of the N-linked glycosylation site may vary by 1, 2 or 3 amino acids in the glycosylation-modified IL-20 and still maintain the desired property of preferentially signaling through one of the IL-20 receptor complexes. Therefore, the invention is contemplated to include those glycosylation-modified IL-20 polypeptides that have at least one N-linked glycosylation sites at amino acids 61-63 (site 1), 97-99 (site 2) and/or 140-142
- 25 (site 3), plus or minus 1, 2, or 3 amino acids for each of the sites, (e.g., amino acid residues 58-60, 59-61, 60-62, 62-64, 63-65 or 64-66 for site 1; 94-96, 95-97, 96-98, 98-100, 99-101 or 100-102 for site 2; 137-139, 138-140, 139-141, 141-143, 142-144 or 143-145 for site 3). The polypeptides of the invention, when inside the cell, may have a signal peptide sequence to enable protein transport within the cell; however, this signal peptide sequence is not present
- 30 in the mature glycosylation-modified IL-20 polypeptide as it exists when outside the cell. The signal peptide may be that as present in the wild type IL-20 polypeptide (amino acid residues 1 to about 24 of SEQ ID NO: 1) or it may be the signal peptide of another

polypeptide operably linked to the mature sequence of IL-20 that begins at about amino acid 25 of SEQ ID NO: 1.

5 A signal peptide, comprised of about 10-30 hydrophobic amino acids, targets the nascent protein from the ribosome to the endoplasmic reticulum (ER). Once localized to the ER, the proteins can be further directed to the Golgi apparatus within the cell. The Golgi distributes proteins to vesicles, lysosomes, the cell membrane, and other organelles. Proteins targeted to the ER by a signal sequence can be released from the cell into the extracellular space. This is the case for glycosylation-modified IL-20 polypeptides of the present invention. For example, vesicles containing proteins to be moved outside the cell can fuse
10 with the cell membrane and release their contents into the extracellular space via a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles until exocytosis is triggered. Proteins that transit through this pathway are either released into the extracellular space or retained in the plasma membrane. The IL-20 polypeptide is released into extracellular space.
15 In many instances the amino acids comprising the signal peptide are cleaved off the protein during transport or once its final destination has been reached. Specialized enzymes, termed signal peptidases, are responsible for the removal of the signal peptide sequences from proteins. These enzymes are activated once the signal peptide has directed the protein to the desired location.

20 A glycosylation-modified IL-20 polypeptide may be produced recombinantly, not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which *e.g.*, may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of an expression vector, or it may be a part of the polypeptide-encoding DNA that is inserted into
25 such a vector. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, aha factor leader (including *Saccharomyces* and *Kluyveromyces* cc-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179), or the signal described in International Patent Publication No. WO 90/13646. In mammalian cell expression, mammalian signal sequences may be
30 used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species as well as viral secretory leaders.

Also provided by the present invention are nucleic acid molecules comprising polynucleotides encoding the polypeptides. Exemplary polynucleotides are shown in SEQ ID NOs: 14-17. Also provided are polynucleotide variants that are homologous or substantially identical to SEQ ID NOs: 14-17.

5

Variants

The present invention encompasses variants of a polynucleotide sequence encoding a glycosylation-modified IL-20 polypeptide, or variant polypeptide thereof, disclosed in SEQ ID NOs: 10-13 and their complementary strands. The present invention also encompasses variants of the glycosylation-modified IL-20 polypeptide sequences disclosed in SEQ ID NOs: 5-8 and 14-21. The term "variant" refers to a polynucleotide or polypeptide differing from a polynucleotide sequence or a polypeptide sequence as shown in the SEQ ID NOs of the present invention (respectively), but retaining essential properties thereof such as a particular activity or function of interest. For example, a variant glycosylation-modified IL-20 polypeptide encompassed within the scope of the invention would preferentially signal through one IL-20 receptor complex at a level greater than through another IL-20 receptor complex, substantially similar to the non-variant form of the glycosylation-modified IL-20 polypeptide. Generally, variants are closely similar overall in structural and/or sequence identity, and, in many regions, identical to a polynucleotide or polypeptide of the present invention. The term "variant" is further described in the definitions herein.

The present invention encompasses nucleic acid molecules that comprise or alternatively consist of, a polynucleotide sequence that is at least 95%, 96%, 97%, 98%, or most preferably at least 99% identical to a polynucleotide comprising the sequence of SEQ ID NOs: 10-13 (or a strand complementary thereto); or a polynucleotide sequence encoding a polypeptide that is at least 95%, 96%, 97%, 98%, or most preferably at least 99% identical to that of SEQ ID NO: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 or 21 in which the NX(T/S) sequence encoding the N-linked glycosylation site may be shifted 1, 2, or 3 amino acids in either direction and still fall within the scope of the invention.

30

The present invention is also directed to polypeptides that comprise, or alternatively consist of, an amino acid sequence that is at least: 95%, 96%, 97%, 98%, or 99% identical to a polypeptide sequence of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19,

20 or 21 in which the NX(T/S) sequence encoding the N-linked glycosylation site may be shifted 1, 2, or 3 amino acids in either direction and still fall within the scope of the invention.

A polynucleotide sequence having at least some "percentage identity," (e.g., 95%)
5 to another polynucleotide sequence, means that the sequence being compared (e.g., the test sequence or candidate sequence) may vary from another sequence (e.g. the reference sequence) by a certain number of nucleotide differences (e.g., a test sequence with 95% sequence identity to a reference sequence can have, on average, up to five point mutations per each 100 contiguous nucleotides of the referent sequence). In other words, for a test
10 sequence to exhibit at least 95% identity to a reference sequence, up to 5% of the nucleotides in the reference may differ, e.g., be deleted or substituted with another nucleotide, or a number of nucleotides (up to 5% of the total number of nucleotides in the reference sequence) may be inserted into the reference sequence. As a practical matter, determining if a particular nucleic acid molecule or polynucleotide sequence exhibits at
15 least about: 95%, 96%, 97%, 98% or 99% identity to a polynucleotide sequence can be accomplished using known computer programs. Algorithms for sequence analysis are known in the art, such as BLAST, described in Altschul *et al.*, J Mol Biol 215:403, 1990.

Especially preferred are polynucleotide variants containing alterations, which produce silent substitutions (*i.e.*, no change in amino acid encoded thereby), additions, or
20 deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred.

A further indication that two nucleic acid sequences of polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is
25 immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described herein. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as
30 described below.

A polypeptide exhibiting or having at least about, e.g., 95% "sequence identity" to another amino acid sequence may include, e.g., up to five amino acid alterations per each

100 amino acid (on average) stretch of the test amino acid sequence. In other words, a first amino acid sequence that is at least 95% identical to a second amino acid sequence, can have up to 5% of its total number of amino acid residues different from the second sequence, e.g., by insertion, deletion, or substitution of an amino acid residue.

5 Alterations in amino residues of a polypeptide sequence may occur, e.g., at the amino or carboxy terminal positions or anywhere between these terminal positions, interspersed either individually among residues in the sequence or in one or more contiguous sections, portions, or fragments within the sequence.

10 When determining percent identity between a reference polypeptide (e.g., Gly1 as shown in SEQ ID NO: 5) and a fusion protein in which the reference polypeptide is joined to, e.g., an affinity tag, (e.g., Gly1 fused to FLIS tag as discussed in the Examples); it is not intended in the present invention that the portion of the fusion protein that does not encode a glycosylation-modified IL-20 (in this example, the FLIS tag) be included in the alignment or the percent identity analysis, but rather, only that portion of the fusion
15 protein encoding a glycosylation-modified IL-20 should be included in the alignment to the reference polypeptide.

20 Variants encompassed by the present invention may contain alterations in the coding regions, non-coding regions, or both. Moreover, variants in which 1-2, 1-5, or 5-10 amino acids are substituted, deleted, or added in any combination are preferred. Even more preferably the variant glycosylation-modified IL-20 polypeptides preferentially signal through one of the IL-20 receptor complexes. The invention encompasses polypeptide variants that show a biological activity of the reference glycosylation-modified IL-20 such as, e.g., ligand binding for an IL-20 receptor complex or antigenicity. Such variants include, e.g., deletions, insertions, inversions, repeats, and substitutions
25 selected so as to have little effect on activity using general rules known in the art. For example, teachings on making phenotypically silent amino acid substitutions are provided, e.g., by Bowie, *et al.*, Science 247:1306, 1990.

30 In order of ever-increasing preference, it is highly preferable for a polypeptide of the invention to have an amino acid sequence that comprises an amino acid sequence of the present invention which contains zero or one, but not more than: 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions; wherein conservative amino acid substitutions are more preferable than non-conservative substitutions.

Expression and Purification of Glycosylation Modified IL-20

Recombinant expression vectors are typically self-replicating DNA or RNA constructs containing a desired gene to be expressed that is operably linked to a promoter and optionally other control elements that will be recognized in a suitable host cell. The specific type of control elements necessary to effect expression depends on the host cell used and the level of expression desired. Proteins of the invention can be expressed in mammalian cells, yeast, insect or other cells under the control of appropriate promoters and which are capable of glycosylating the protein at N-linked glycosylation consensus sites of NX(T/S).

Vectors, as used herein, encompass plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles that enable the integration of DNA fragments into the genome of the host although, optionally, expression can occur transiently without integration. Plasmids are the most commonly used form of vector, but many other forms of vectors that perform an equivalent function are also suitable for use.

Expression and cloning vectors will typically contain at least one selection gene. Expression vectors further contain a promoter operably linked to the polypeptide-encoding nucleic acid sequence to direct mRNA synthesis. A typical mammalian expression vector contains at least one promoter element that mediates initiation of transcription of mRNA, the polypeptide of interest's coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional optional elements include enhancer(s), a Kozak sequence and an intervening sequence (intron) flanked by donor and acceptor sites for RNA splicing. To direct a glycosylation-modified IL-20 polypeptide into the secretory pathway of a host cell, a signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The signal sequence may be that of the IL-20 protein, or may be derived from another secreted protein (e.g., t-PA), or synthesized *de novo*. The secretory signal sequence is joined to the DNA sequence encoding the glycosylation-modified IL-20 in the correct reading frame. Signal sequences are commonly positioned 5' to the DNA sequence encoding the mature polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of foreign polypeptides therein is disclosed in U.S. Patent No. 5,162,222. The use of *Agrobacterium*

rhizogenes as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, J. Biosci. 11:47, 1987. Insect cells can be infected with recombinant baculovirus (see Richardson, C. Ed., *Baculovirus Expression Protocols. Methods in Molecular Biology*, (Humana Press, Totowa, NJ, 1995) or BAC-TO-BAC of Life Technologies.

5 Methods for introducing exogenous DNA into mammalian host cells or other cells are well known in the art and include calcium phosphate-mediated transfection, Wigler, *et al.*, *Cell* 14:725, 1978; electroporation, Neumann *et al.*, EMBO J. 1:841, 1982; DEAE-dextran mediated transfection, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, (John Wiley and Sons, Inc., NY, 1987), and liposome-mediated transfection
10 (e.g., Promega Corp., Mirrus Corp). Alternatively, the nucleic acid encoding the polypeptide of interest is expressed in stable cell lines, cultured mammalian cells that contain the nucleic acid integrated into a host chromosome. The co-transfection of the nucleic acid encoding the polypeptide of interest along with a gene encoding a selectable marker such as DHFR (dihydrofolate reductase), GPT neomycin, or hygromycin allows
15 the identification and isolation of the transfected cells.

 The transfected gene can also be amplified to express large amounts of the encoded polypeptide. The DHFR marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (Murphy, *et al.*, Biochem. J. 227:277, 1991; Bebbington, *et al.*,
20 BioTechnology 10:169, 1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of polypeptides. CHO cells are efficient at producing glycosylated polypeptides.

25 The description below relates primarily to production of a polypeptide of the invention by culturing cells transformed or transfected with a vector containing polypeptide-encoding nucleic acid. It is contemplated that alternative methods, well known in the art, may be used to prepare polypeptides. For instance, the sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (see, e.g., Stewart *et al.*,
30 Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc. 85:2149, 1963). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using

an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of a polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce a full-length.

Host cells are transfected or transformed with expression vectors described herein for polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Suitable host cells for cloning the nucleic acid in the vectors herein include prokaryote, yeast, or higher eukaryote cells, however for expression of glycosylation-modified IL-20, bacteria may not be used. Alternatively, PCR or other nucleic acid polymerase reactions, are suitable. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for the polypeptide expressing vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism.

Suitable host cells for the expression of glycosylated polypeptides of the invention are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sp, *Spodoptera* high5 as well as plant cells. Examples of useful mammalian host cell lines include, e.g., CHO and COS cells.

Polypeptides of the invention may be recovered from culture medium or from host cell lysates and analyzed e.g., for their signaling ability as demonstrated in the Examples herein. It is preferred to purify the polypeptides of the invention to greater than about 80% purity, more preferably to at least 90% purity, even more preferably to at least 95% purity, and free of infectious and pyrogenic agents. The glycosylation-modified IL-20 polypeptides of the present invention are not membrane-bound. Cells employed in expression of polypeptides can be disrupted for protein purification by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

The following procedures are exemplary of suitable purification procedures: fractionation on an ion-exchange column; ethanol precipitation; reversed-phase HPLC;

chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of polypeptides. Various methods of protein purification may be employed and such methods are known in the art and described, for example, in Deutscher, Methods in Enzymology 182:83, 1990 and Scopes, Protein Purification: Principles and Practice, Springer-Verlag, NY (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular polypeptide produced.

Many types of analyses can be performed with the polypeptides of the present invention to demonstrate their role in the development, pathogenesis, and treatment of hematopoietic disorders, cancer, cardiovascular disease, skin disorders and immune related disease. Certain analyses are exemplified in the Examples herein. Animal models can be used to further understand the role of the polypeptides of the invention.

Utility of Glycosylation Modified IL-20

Glycosylation-modified IL-20 polypeptides, and variants thereof, are useful for the prevention, diagnosis, and treatment of cancer, cardiovascular disorders and immune system disorders, skin disorder such as psoriasis, inflammation, obesity, and hematopoietic disorders.

Data presented within the Examples herein demonstrate that glycosylation-modified IL-20 may be expressed and glycosylated in mammalian cells and secreted therefrom (Examples 2 and 3). The data further demonstrate that glycosylation-modified IL-20, as exemplified by Gly1, Gly3 and Gly4, may preferentially signal through one of the multiple IL-20 receptor complexes (Example 5).

Particular cancers suitable for treatment with the polypeptides of the invention include, but are not limited to, acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia and chronic granulocytic leukemia. Additional cancers suitable for treatment with the polypeptides of the invention include, but are not limited to, adenocarcinoma, lymphoma,

melanoma, myeloma, Hamartoma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

5 Particular cardiovascular disorder suitable for treatment with the polypeptides of the invention include, but are not limited to, congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective
10 endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, congenital heart disease, complications of cardiac transplantation, arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors,
15 and complications of thrombolysis, balloon angioplasty, vascular replacement, and coronary artery bypass graft surgery.

Particular immune system disorders suitable for treatment with the polypeptides of the invention include, but are not limited to, inflammatory disorders, acquired
immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome,
20 allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic
25 gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis,
30 thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma.

Particular hematopoietic disorders suitable for treatment with the polypeptides of the invention include, but are not limited to, various diseases arising from imbalances between degradation and reconstitution of blood cells or from generation of inappropriate numbers of certain types of blood cells and requiring enhancing or stimulating of hematopoiesis, erythropoiesis, leukopoiesis, thrombocytopoiesis, production of neutrophils, granulocytes, and/or platelets by stimulating the proliferation and/or differentiation of progenitors of such cells, as needed in various conditions and/or situations, including, but not limited to, the following:

(a) inadequate platelet production, such as aplastic anemia, refractory anemias, leukemia, preleukemia/ myelodysplastic syndromes, megaloblastic anemia, chemotherapy or radiation therapy, and existing platelet deficiency or an expected platelet deficiency (e.g., because of planned surgery including, but not limited to, organ/bone marrow transplantations);

(b) increased destruction of platelets, such as idiopathic thrombocytopenia purpura, other immune thrombocytopenias, IV-associated thrombocytopenia, sepsis/disseminated intravascular coagulation, and vasculitis;

(c) abnormal platelet function, such as Glanzmann's thrombasthenia, acute/chronic leukemia, myeloproliferative disorders, uremia, platelet storage pool disease, Von Willebrand disease, and postoperative cardiovascular dysfunction, and

(d) other blood coagulation disorders such as afibrinogenemia or wounds of any origin.

Particular skin disorders suitable for treatment with the polypeptides of the invention include, but are not limited to, chronic skin inflammation, psoriasis, erythema, keratinocyte activation, eczema, wound healing or dry skin in general.

As used herein, the term "CFU-GEMM stimulating amount" when referring to a glycosylation-modified IL-20 polypeptide refers to an amount of glycosylation-modified IL-20 that raises the baseline number of CFU-GEMM cells by at least 20%, preferably 30%, 40%, 50%, 60%, 70%, 80% or greater over the number of CFU-GEMM cells present in a mammal, human, or patient in the absence of glycosylation-modified IL-20. As used herein, the term "hematopoietic progenitor stimulating amount" when referring to glycosylation-modified IL-20 refers to an amount of glycosylation-modified IL-20 that raises the baseline number of any hematopoietic progenitor cell type by at least 20%, preferably 30%, 40%,

50%, 60%, 70%, 80% or greater over the number of that type of hematopoietic progenitor cells present in a mammal, human, or patient in the absence of glycosylation-modified IL-20.

When the coding sequence for a glycosylation-modified IL-20 polypeptide encodes a protein which binds to another protein as is the case for extracellular glycosylation-modified IL-20 of the present invention, the glycosylation-modified IL-20 polypeptide can be used in assays to identify the other proteins or molecules involved in the binding interaction or subsequent signaling response. By such methods, inhibitors of the receptor/ligand binding interaction or signaling can be identified. Proteins involved in such binding interactions or signaling can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction or signaling. Also, the receptor polypeptide can be used to isolate correlative ligand(s).

The active agents of the present invention are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebral, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, intraocular, intralesional, oral, topical, inhalation, pulmonary, and/or through sustained release.

Other therapeutic regimens may be combined with the administration of a polypeptide of the invention. For the prevention or treatment of disease, the appropriate dosage of an active agent will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. An effective amount of at least one of the glycosylation-modified IL-20 in combination with a pharmaceutically acceptable sterile vehicle may be determined as described, for example, in Remington's Pharmaceutical Sciences; *Drug Receptors and Receptor Theory*, 18th ed., Mack Publishing Co., Easton, Pa. (1990). Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down

by Mordenti and Chappell, "The Use of Interspecies Scaling in Toxicokinetics," in *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds., Pergamon Press, NY 1989, pp.4246.

Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760, 5,206,344 or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is readily monitored by conventional techniques and assays.

Compositions of the invention may further contain common carriers and excipients such as for example, corn-starch or gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid.

Compositions to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes).

Examples

General Methods

Commercially available reagents referred to in the examples are used according to manufacturer's instructions unless otherwise indicated. The present invention uses standard procedures of recombinant DNA technology such as those described or referenced in Sambrook, *et al.*, (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, NY; Ausubel, *et al.* (1989 and supplements), *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, NY; Innis *et al.*, unless otherwise noted.

30 Example 1 Mutagenesis and Cloning of Glycosylation-Modified IL-20

Site directed mutagenesis was performed using a nucleic acid encoding a FLIS-tagged (FLAG + HIS6) wild-type IL-20 to generate four different IL-20 glycosylation

mutants named Gly1, Gly2, Gly3 and Gly4 (Fig. 1). The nucleic acid encoding IL-20, in a pGEM plasmid (Promega Corp.), was mutagenized using QUIKCHANGE™ Site-Directed Mutagenesis Kit (Stratagene) using the method described by the manufacturer. The sequence encoding the FLIS tag was present at the 3' end of the FLIS-tagged IL-20 gene to accommodate the purification of the protein when expressed; however, it is not necessary for function of the protein.

Gly1 is IL-20 altered such that the lysine residue at position 141 in the wild type IL-20 protein is replaced with an asparagine, the lysine residue at position 142 in the wild type protein is replaced with an alanine, and the tyrosine residue at position 143 in the wild type protein is replaced with a threonine, thereby creating an N-linked glycosylation sequence (NAT) at the equivalent position where the second N-linked glycosylation sequence (NAT) exists in IL-19 (see Fig. 1).

Gly2 is IL-20 altered such that an asparagine-arginine pair of residues is inserted between the glutamine residue at position 99 and the threonine residue at position 100 of the wild type IL-20 protein, thereby creating an N-linked glycosylation sequence (NRT) at the equivalent position where an NRT glycosylation sequence is located in IL-24 and creating a protein that is two amino acids larger than the wild type IL-20 protein (Fig. 1).

Gly3 is IL-20 altered such that the tyrosine residue at position 98 and the glutamine at position 99 of the wild type IL-20 protein are replaced with an asparagine-arginine pair of residues, thereby creating an N-linked glycosylation sequence (NRT) at the equivalent position where an NRT glycosylation sequence is located in IL-24 and creating a protein that is identical in size to the wild type IL-20 (see Fig. 1).

Gly 4 is IL-20 altered such that the aspartic acid residue at position 61 in the wild type IL-20 protein is replaced with an asparagine, the isoleucine residue at position 62 in the wild type protein is replaced with a valine and the arginine residue at position 63 in the wild type protein is replaced with a threonine, thereby creating an N-linked glycosylation sequence (NVT) at the same location where the first N-linked glycosylation sequence (NVT) exists in IL-19 (see Fig. 1).

The following show the sequences of the open reading frames encoding the wild type or mutant IL-20, the amino acid sequence of the wild type or mutant IL-20, wild type IL-10, and the receptor subunits used by IL-20, with the symbol (*) indicating either a stop codon or additional sequence encoding a polypeptide (or stop signal or the amino

- acid sequence of fusion protein for the amino acid sequences) operably linked to the upstream sequence, e.g., an affinity tag useful for purification. These sequences do not include the optional FLIS tag encoded by 5' gatatcgactacaaggatgacgacgacaagcacgtgcatcaccatcaccatcactag 3' (SEQ ID NO: 22). The consensus N-linked glycosylation domain of NXT/S is underlined. N represents asparagine, X is any amino acid and T/S is either a threonine or a serine.

IL-20 wild type ORF (SEQ ID NO: 9)

- 10 atgaaagcctctagtcctgccttcagccttctctctgctgcgttttatctcctatggact
ccttccactggactgaagacactcaatttggaagctgtgtgatcgccacaaaccttcag
gaaatacgaaatggattttctgagatacggggcagtggtgcaagccaaagatggaaacatt
gacatcagaatcttaaggaggactgagtcctttgcaagacacaaagcctggaatcgatgc
tgccctcctgcgccatttgctaagactctatctggacagggattttaaaaactaccagacc
cctgaccattatactctccggaagatcagcagcctcgccaattcctttcttaccatcaag
15 aaggacctccggctctgtcatgcccacatgacatgccattgtggggaggaagcaatgaag
aaatacagccagattctgagtcactttgaaaagctggaacctcaggcagcagttgtgaag
gctttgggggaactagacattcttctgcaatggatggaggagacagaa*

Gly1 polynucleotide sequence (SEQ ID NO: 10)

- 20 atgaaagcctctagtcctgccttcagccttctctctgctgcgttttatctcctatggact
ccttccactggactgaagacactcaatttggaagctgtgtgatcgccacaaaccttcag
gaaatacgaaatggattttctgagatacggggcagtggtgcaagccaaagatggaaacatt
gacatcagaatcttaaggaggactgagtcctttgcaagacacaaagcctggaatcgatgc
tgccctcctgcgccatttgctaagactctatctggacagggattttaaaaactaccagacc
25 cctgaccattatactctccggaagatcagcagcctcgccaattcctttcttaccatcaag
aaggacctccggctctgtcatgcccacatgacatgccattgtggggaggaagcaatgaac
gcaactagttagattctgagtcactttgaaaagctggaacctcaggcagcagttgtgaag
gctttgggggaactagacattcttctgcaatggatggaggagacagaa*

30 Gly2 polynucleotide sequence (SEQ ID NO: 11)

- atgaaagcctctagtcctgccttcagccttctctctgctgcgttttatctcctatggact
ccttccactggactgaagacactcaatttggaagctgtgtgatcgccacaaaccttcag
gaaatacgaaatggattttctgagatacggggcagtggtgcaagccaaagatggaaacatt

29

gacatcagaatcttaaggaggactgagtcctttgcaagacacaaagcctgcgaatcgatgc
tgccctcctgcgccatttgctaagactctatctggacagggatatttaaaaactaccagaat
agaacccctgaccattatactctccggaagatcagcagcctcgccaattcctttcttacc
atcaagaaggacctccggctctgtcatgcccacatgacatgccattgtggggaggaagca
5 atgaagaaatacagccagattctgagtcactttgaaaagctggaacctcaggcagcagtt
gtgaaggctttgggggaactagacattcttctgcaatggatggaggagacagaa*

Gly3 polynucleotide sequence (SEQ ID NO: 12)

atgaaagcctctagtcttgccctcagccttctctctgctgcggtttatctcctatggact
10 ccttccactggactgaagacactcaatttggaagctgtgtgatcgccacaaaccttcag
gaaatacgaaatggattttctgagatacggggcagtggtgcaagccaaagatggaaacatt
gacatcagaatcttaaggaggactgagtcctttgcaagacacaaagcctgcgaatcgatgc
tgccctcctgcgccatttgctaagactctatctggacagggatatttaaaaacagaacccct
gaccattatactctccggaagatcagcagcctcgccaattcctttcttaccatcaagaag
15 gacctccggctctgtcatgcccacatgacatgccattgtggggaggaagcaatgaagaa
tacagccagattctgagtcactttgaaaagctggaacctcaggcagcagttgtgaaggct
ttgggggaactagacattcttctgcaatggatggaggagacagaa*

Gly4 polynucleotide sequence (SEQ ID NO: 13)

atgaaagcctctagtcttgccctcagccttctctctgctgcggtttatctcctatggact
20 ccttccactggactgaagacactcaatttggaagctgtgtgatcgccacaaaccttcag
gaaatacgaaatggattttctgagatacggggcagtggtgcaagccaaagatggaaacatt
aacgtcacaatattaaggaggactgagtcctttgcaagacacaaagcctgcgaatcgatgc
tgccctcctgcgccatttgctaagactctatctggacagggatatttaaaaactaccagacc
25 cctgaccattatactctccggaagatcagcagcctcgccaattcctttcttaccatcaag
aaggacctccggctctgtcatgcccacatgacatgccattgtggggaggaagcaatgaag
aaatacagccagattctgagtcactttgaaaagctggaacctcaggcagcagttgtgaag
gctttgggggaactagacattcttctgcaatggatggaggagacagaa*

30 IL-20 polypeptide sequence (SEQ ID NO: 1)

mkasslafslsaa fyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
dirilrrteslqdtkpanrccllrhlrllyldrvmknyqtpdhytlrkisslansfltik
kdrlrlchahmtchcgeeammkysqilshfeklepqaavvkalgeldillqwmeete*

IL-10 polypeptide sequence (SEQ ID NO: 4)

mhssallcclvlltgvrspgqgtqsenscthfpgnnmlrdlrdafsrvtffqmkdqld
nlllkeslledfkgylgcqalsemiqfyleevmpqaenqdpdikahvnsngenlktlrir
lrrchrfcenkskaveqvknafnklqekgiykamsefdefinyieaymtmkirn*

5

Gly1 polypeptide sequence (SEQ ID NO: 5)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
dirilrrteslqdtkpanrccllrhlrllyldrvmknyqtpdhytlrkisslansfltik
kdrlrchahmtchcgeeamnatssqilshfeklepqaavvkalgeldillqwmeete*

10

Gly2 polypeptide sequence (SEQ ID NO: 6)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
dirilrrteslqdtkpanrccllrhlrllyldrvmknyqrtpdpdhytlrkisslansfltik
ikkdlrchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwmeete*

15

Gly3 polypeptide sequence (SEQ ID NO: 7)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
dirilrrteslqdtkpanrccllrhlrllyldrvmknrtpdhytlrkisslansfltik
dlrlchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwmeete*

20

Gly4 polypeptide sequence (SEQ ID NO: 8)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
nvtilrrteslqdtkpanrccllrhlrllyldrvmknyqtpdhytlrkisslansfltik
kdrlrchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwmeete*

25

Generic Gly1 polypeptide sequence (SEQ ID NO: 14)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
dirilrrteslqdtkpanrccllrhlrllyldrvmknyqtpdhytlrkisslansfltik
kdrlrchahmtchcgeeamnx(t/s)sqilshfeklepqaavvkalgeldillqwmeete

30

*

Generic Gly2 polypeptide sequence (SEQ ID NO: 15)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
 dirilrrteslqdtkpanrccllrhlrllyldrvfk~~nx~~(t/s)pdhytlrkisslan
 5 sfltikkdlrlchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwmeete*

Generic Gly3 polypeptide sequence (SEQ ID NO: 16)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
 10 dirilrrteslqdtkpanrccllrhlrllyldrvfk~~nx~~(t/s)pdhytlrkisslansfl
 tikkdrlrlchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwmeete*

Generic Gly4 polypeptide sequence (SEQ ID NO: 17)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
 15 ~~nx~~(t/s)ilrrteslqdtkpanrccllrhlrllyldrvfknyqtpdhytlrkisslansf
 ltikkdlrlchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwmeete
 *

Generic Gly1 plus Gly4 polypeptide sequence (SEQ ID NO: 18)

20 mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
~~nx~~(t/s)ilrrteslqdtkpanrccllrhlrllyldrvfknyqtpdhytlrkisslansf
 ltikkdlrlchahmtchcgeeam~~nx~~(t/s)sqilshfeklepqaavvkalgeldillqwm
 eete*

Generic Gly1 plus Gly3 polypeptide sequence (SEQ ID NO: 19)

25 mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
 dirilrrteslqdtkpanrccllrhlrllyldrvfk~~nx~~(t/s)pdhytlrkisslansf
 tikkdrlrlchahmtchcgeeam~~nx~~(t/s)sqilshfeklepqaavvkalgeldillqwm
 ete*

30

Generic Gly3 plus Gly4 polypeptide sequence (SEQ ID NO: 20)

mkasslafslsaaafyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
~~nx~~(t/s)ilrrteslqdtkpanrccllrhlrllyldrvfk~~nx~~(t/s)pdhytlrkissla

nsfltikkdlrlchahmtchcgeeamkkysqilshfeklepqaavvkalgeldillqwme
ete*

Generic Gly1 plus Gly3 plus Gly4 polypeptide sequence (SEQ ID NO: 21)

5 mkasslafslsaa fyllwtpstglktlnlgscviatnlqeirngfseirgsvqakdgni
nx(t/s)ilrrteslqdtkpanrccllrhlrllyldrvfknx(t/s)pdhytlrkissla
nsfltikkdlrlchahmtchcgeeamnx(t/s)sqilshfeklepqaavvkalgeldill
qwmeete*

10 IL20R1 (SEQ ID NO: 22)

MRAPGRPALRPPLLLLLLAAPWGRAVPCVSGGKPANITFLSINMKNVLQWTPPE
GLQGVKVITYTVQYFIYGQKKWLNKSECRNINRTYCDLSAETSDYEHQYYAKVKAIWGTKC
SKWAESGRFYPFLETQIGPPEVALTTDEKSISVVLTAPEKWKRNPEDVSMQQIYSNLKYN
VSVLNTKSNRTWSQCVTNHTLVLTWLEPNTLYCVHVESFVPGPPRAQPSEKQCARTLKD
15 QSSEFKAKIIFWYVISITVFLFSVMGYSIYRIHVGKEKHPANLILYIGNEFDKRFFVPA
EKIVINFITLNISSDKISHQDMSLLGKSSDVSSLNDPQPSGNLRPPQEEEEVKHLGYAS
HLMEIFCDSEENTEGTSLTQQESLSRTIPDKTVIEYEDVRTTDICAGPEEQELSLQEE
VSTQGTLLSQAALAVLGPQTLQYSYTPQLQDLPLAQEHTDSEEGPEEPSTTLVDWDP
QTGRLCIPSLSSFDQDSEGCEPSEGDLGEEGLLSRLYEPPAPDRPPGENETYLMQFMEE

20 WGLYVQMEN*

IL20R2 (SEQ ID NO: 3)

MQTFTMVLEEIWTSLFMWFFYALIPCLLTDEVAILPAPQNLSVLSTNMKHLMLWSPVIAP
GETVYYSVEYQGEYESLYTSHIWIWIPSSWCSLTEGPECDVTDDITATVPYNLRVRATLGSQ
25 TSAWSILKHPFNRNSTILTRPGMEITKDGFLVIELEDLGPQFEFLVAYWRREPGEAEHV
KMVRSGGIPVHLETMEPGAAYCVKAQTFVKAIGRYSAFSQTECEVQGEAIPLVLALFAF
VGFMLILVVVPLFVWKMGRLLOYSCCPVVVLPTLKITNSPQKLISCRREEVDACATAVM
SPEELLRAWIS

30 IL22R1 (SEQ ID NO: 2)

mrtlltiltvgsaahapedpsdllqhvkfqssnfeniltwdsgpegtpdtvysieykty
gerdwvakkgcqritrksnltvetgnltelyyarvtavsaggrsatkmdrfsslqhtt
lkppdvtciskvrsiqmivhptptpiragdghriltledifhdfyhlelqvnrtymhlq
gkqreyeffgltpdteflgtimicvptwakesapymcrvktdrwtysfsgaflfsmgfl

vavlcylsyryvtkppappnslnvqrvtlfgplrfiqehvlipvfdlsgpsslaqpvyys
 qirvsgprepagapqrhslseitylgqpdilqpsnvpppqilsplsyapnaapevgpp
 syapqvtpeaqfpfyapqaiskvqpssyapqatpdswwpsygvcmegsgkdsptgtlssp
 khlrpkqqlqkeppagscmlggslqevtslameesqeakslhqplgictdrtsdpnlh
 5 sgeegtpqylkgqllssvqieghpmslqppsgpcspdqgppspwgllslvcpkdeaksp
 apetsdleqpteldslfrglaltvqwes*

The mutant polynucleotide sequences were verified and then transferred from the pGEM vector to an expression vector. Any expression vector containing a promoter
 10 sequence functional in mammalian cells, yeast, or baculovirus could be used to express the glycosylated proteins of the present invention.

Example 2 Transient transfections of 293 EBNA cells

For small-scale transient transfections, 293 EBNA cells (Invitrogen) were grown
 15 in a T75 cm² flask in DMEM/F12 supplemented with 10% (v/v) fetal bovine serum (FBS) 37°C. After PBS washing, the cells were trypsinized and resuspended to a concentration of 0.5 x 10⁶ cells/ml. To each well of a Biocoat Poly-D-Lysine Plate 6 plate (Fisher, Becton Dickinson) was added 1 ml of cells and an additional 1.5 mL of DMEM/F12 (3:1) medium supplemented with 10% (v/v) serum, 20 mM HEPES and 50 µg/ml of
 20 gentamicin. Cells were left to recover until attaining approximately 70-80% confluence, at which point the growth media was aspirated and replaced with 1 ml of serum free medium containing 20 mM HEPES (20 ml/L), insulin-transferrin-selenium (10 ml/L), 2 mM L-glutamine and 50 µg/ml of gentamicin. In a separate tube was added 80 µl of OPTI-Medium and 5 µl of FuGENE (Roche). After thorough mixing and a 5 minute
 25 incubation at 22°C, 3 µg of IL-20 DNA in the expression vector described in Example 1 (or IL-20 glycosylation-mutant DNA) was added and the mixture incubated at 22°C for 20 min. The media/DNA mix was then added to each well of the 6 well plate containing cells. After 24 hour incubation at 37°C, the media was removed and replaced with serum-free medium containing 20 mM HEPES (20 ml/L), insulin-transferrin-selenium (10
 30 ml/L), 2 mM L-glutamine and 50 µg/ml of gentamicin. Media from these transfections was analyzed by Western Blot (Example 3) to determine whether the mutant proteins were expressed, secreted and glycosylated.

For large scale transient transfections, HEK293EBNA cells, adapted to suspension culture in animal protein-free medium (AFPM), are grown in spinner flasks. Cells are maintained in 20-liter flasks (130 rpm, 37°C) at a working volume of 9.5 liters and a cell density between 0.6×10^6 cells/ml. The transfection reagent, X-TREMEGENE (Roche Diagnostics), is used to introduce the expression vector into the cells. Then, 2.5 mg of the appropriate plasmid DNA is added to 500 ml of basal AFPM followed by the addition of 5 ml of transfection reagent. After a 30 minute incubation, the 500 ml DNA/reagent complex is added to the Bellco reactor. The Bellco is run under agitation for 5 days at 37°C and controlled at a maximum pH of 7.1 and a dissolved oxygen level of 50% saturation. On day 5, cells are removed by centrifugation at 3000 rpm for 30 minutes, and the supernatant is submitted for purification as described in Example 4 belowwherein.

293EBNA suspension medium ingredients (AFPM):

- DME/F12 powder (formula 82-5033EJ, GibcoBRL) - - 14.98 g/l
- Sodium bicarbonate - - 2.2 g/l
- Pluronic F-68, 0.05% (Sigma P1300) - - 0.8 g/l
- Human recombinant insulin (Nucellin, Lilly) - - 5 mg/l
- Tropolone (Sigma T7387) - - 0.4 mg/l

Example 3 Western Blot Analysis

Western blot analysis was performed on media from transiently transfected cells described in Example 2 to detect secreted IL-20 wild-type and glycosylation-mutant polypeptides. 100 µl of media was removed from the transfected cells and added to a tube containing 100 µl of 2X sample buffer (Novex, Tris-glycine SDS sample buffer). Samples were heated at 100°C, 3 min., and loaded onto a Tris-glycine SDS gel. Electrophoresis was performed and the protein transferred onto 0.2 µm nitrocellulose. Membranes were rinsed in PBS supplemented with 0.1 % v/v Tween-20 (PBST) prior to blocking for 16 hour at 4°C in PBS/5 % milk. Membranes were incubated with the primary antibody that reacts to the FLAG affinity tag that was fused to the carboxy terminus of the IL-20 protein, anti-FLAG M2-HRP fused antibody (Sigma, St. Louis, MO) diluted to 1:5,000 in PBST/0.5 % w/v milk for 1 hour at 22°C. Membranes were incubated 1 hour with secondary antibody, anti-mouse HRP (Pierce, Rockford, IL) diluted

1:50,000 in PBST/0.5 % w/v milk. Membranes were rinsed twice in PBST for 10 min each prior to detection with SuperSignal West Pico kit (Pierce, Rockford, IL).

5 Secretion levels of the proteins were detected analyzing media from the transiently transfected cells, while the expression levels of the proteins were detected in cell lysates after removal of the media. Qualitative analysis of the Western blot bands related to standard proteins with known expression profiles was performed. The data demonstrated that all of the proteins were expressed in relatively equal amounts in the transiently transfected cells, but the secretion of each protein (IL-20, IL-19, IL-24 and the four glycosylation mutants Gly1-4) varied. The relative secretion profile for the proteins are as
10 followed: IL19 > IL-20=Gly1=Gly4 > IL-24=Gly2=Gly3. Though there was a variation of the relative levels of secreted protein for each protein, it does not appear to affect the signal strength of each protein in the Pan-STAT luciferase assay (Example 5) since IL-24 always gives equal or greater signal when compared to IL-20 and IL-19.

15 The presence of glycosylation of the Gly1-4 proteins was confirmed by observing an upward size shift of the protein band on the western blot along with the change of a previously tight band (i.e. wild-type IL-20) into a fuzzy band (i.e. the Gly1-4 mutants). This fuzziness is a classic sign of protein glycosylation indicating a heterologously sized product due to the various numbers of sugar moieties added. Glycosylation of Gly4 was further confirmed by mass spec analysis.

20

Example 4 Polypeptide Purification

Cell culture media containing the polypeptide of interest is concentrated in an Amicon ProFlux M12 tangential filtration system using an Amicon S3Y10 UF membrane. The concentrated media is passed over an immobilized metal-affinity chromatography
25 column (Pharmacia) at a flow rate of 2 ml/min. The column is washed with buffer A (PBS (1 mM potassium phosphate, 3 mM sodium phosphate), 0.15 M NaCl, pH 7.4 containing 50 mM Imidazol) until the absorbance returns to baseline. The bound polypeptides are eluted with a gradient from 100% Buffer A to 55% Buffer A developed over 70 min. The gradient is then stepped to 100% Buffer B (buffer A containing 0.5 M
30 Imidazol) for 20 min. Fractions containing the polypeptide of interest are pooled and concentrated using an Ultrafree centrifugal filter unit (Millipore, 10 kDa molecular weight cut-off) to 14 ml. This material is passed over a Superdex 75 (Pharmacia, 26/60) sizing

column equilibrated with PBS, 0.5 M NaCl, pH 7.4, at a flow rate of 3 ml/min. Fractions containing the polypeptide of interest are analyzed by SDS-PAGE.

Example 5 Analysis of glycosylated-mutants of IL-20

5 a. IL-20 Bioassay

This assay demonstrated that the Gly4 mutant of IL-20, which has one of the two IL-19 N-linked glycosylation sites, preferentially signaled through one receptor, the same receptor used by IL-19 (IL20R1/IL20R2). Bone marrow derived BAF/3 cells (Palacios, and Steinmetz, Cell, 1985, 41:727-734) stably transfected to express various combinations of IL-20-Receptor subunits (IL20R1 and IL20R2 or IL20R2 and IL22R1) thereby making them dependent upon IL-20 were maintained in RPMI 1640 supplemented with 10% FBS and 200 ng/ml IL-20. The original synthesis of these stable cell lines was described in U.S. patent application number 60/342,233 entitled "Soluble Proteins that Inhibit Cytokine Signal Transduction Pathways", filed on October 22, 2001 and incorporated herein for the construction of this cell line. The cells were washed twice by centrifuging at 1000 rpm in a Jouan CR422 centrifuge and resuspended in phenol-red-free RPMI 1640 media supplemented with 5% FBS at a concentration of 0.4×10^6 cells/ml. To each well of a 96 well plate was added 50 μ l of cells followed by 50 μ l of purified FLIS-tagged Gly4 mutant IL-20 or wild-type IL-20 (0 to 1000 ng/ml). After 20 hours, 25 μ l of CELLTITER™ 96 Aqueous solution (Promega Corp) was added and after a further 3 hours incubation the plate was read on an ELISA plate reader at 490 nM. The results (mean of at least 4 replicate measurements with the standard deviation (S. Dev.)) provided below in Tables 1 and 2 clearly demonstrate that introduction of an N-linked glycosylation site into IL20 (Gly4) at the position equivalent to the first such site in IL-19, does not adversely influence the ability of this IL-20 mutant (Gly4) to signal through the IL20R1/ IL20R2 receptor complex. However, this mutation attenuates the ability of Gly4 to activate the IL20R2/IL22R1 receptor complex. Thus, the Gly4 IL-20 glycosylation mutant shows preferential receptor specificity. By inserting the IL-19 NVT N-linked glycosylation site into IL-20, the resulting mutant acts more like IL-19 in that it signals better through the IL20R1/ IL20R2 receptor complex than through the IL20R2/IL22R1 receptor complex.

Table 1 IL20R1/IL20R2 cell line

	Ligand conc. (ng/ml)	IL-20 (Mean)	IL-20 (S. Dev.)	Gly4 (Mean)	Gly 4 (S. Dev.)
5	0.0	0	9.44	0	8.35
	0.1	0	1.1	6.19	10.03
	1.0	41.12	5.94	41.69	17.95
	3.0	67.08	3.11	69.76	8.85
	10.0	80.3	4.95	97.43	24.13
10	30.0	82.1	8.25	96.23	27.94
	100.0	92.9	5.48	91.07	15.8
	1000.0	95.71	1.13	101.26	2.54

Table 2 IL22R1/IL20R2 cell line

	Ligand conc. (ng/ml)	IL-20 (Mean)	IL-20 (S. Dev.)	Gly4 (Mean)	Gly 4 (S. Dev.)
15	0.0	0	2.02	0	1.21
	0.1	-2.06	4.52	7.7	16.77
	1.0	39.54	8.42	7.65	5.61
20	3.0	54.97	5.22	15.84	5.86
	10.0	79.77	9.42	14.51	4.82
	30.0	99.77	15.87	10.06	5.38
	100.0	95.18	6.17	16.08	4.27
	1000.0	99.32	2.85	28.63	5.02

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b. Pan-STAT Luciferase Assay

Transcriptional assays were conducted by transient transfection of 293 EBNA cell lines (Edge Biosystems) using a STAT3-luciferase reporter plasmid (Clontech). This assay works by expression of IL-20 or a mutant form of IL-20 which, if it binds to the IL-20 receptor also being expressed in the cells, signals through the Stat3 pathway and upregulates expression of luciferase. The cells were plated at ~50-60% confluency 24 hours pre-transfection in poly-lysine coated 96 well plates with DMEM-F12/10% FBS

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(Gibco). The DNA-liposome complexes were created in OptiMEM (Gibco) media using Lipofectamine 2000 (Gibco) according to manufacturer's instructions and applied to the cells in a 100 µl volume in the absence of serum. Two subunits of the receptor (IL20R1 and IL20R2 or IL22R1 and IL20R2) were also co-transfected into the cells at equal DNA concentrations (10ng each) and the STAT-luciferase plasmid was present in the transfection solution at 100ng. A DNA construct for a ligand protein (IL-20, IL-19, Gly1, Gly2, Gly3 or Gly 4) was added to the transfection solution at 1ng per well along with the other DNAs. Thus, a transfection DNA solution would have four DNAs: (1) the STAT-luciferase plasmid, (2 and 3) two expression vectors, one for each of the two IL-20 receptor subunits to be studied, and (4) an expression vector capable of expressing a ligand protein of interest. The ligand genes used for this experiment were FLIS-tagged. The stimulation was stopped on day three by aspirating the media and adding 75 µl of Glo lysis buffer (Promega). Cells were lysed at room temperature for five minutes before 70 µl of each sample was transferred to a white plate. The plate had 70 µl of BRIGHT-GLO™ reagent added to each well and the two volumes were mixed by pipet and allowed to incubate about 5 minutes before being read on a Luminoskan luminometer. Samples were read at normal gain for 10 seconds/well. All data points are done in 4-8 replicates, the average is reported hereinbelow. The data shown in Table 4 demonstrates that the Gly1, Gly3 and Gly4 mutant IL-20s preferentially signal through the IL20R1/IL20R2 receptor complex as does IL-19.

Table 4 Pan-Stat assay

	Receptor	Ligand	average
	IL20R1/IL20R2	none	25.53
25	IL20R1/IL20R2	IL-20	387.10
	IL20R1/IL20R2	IL-19	392.35
	IL20R1/IL20R2	IL-24	357.08
	IL20R1/IL20R2	Gly1	224.41
	IL20R1/IL20R2	Gly2	383.14
30	IL20R1/IL20R2	Gly3	116.94
	<u>IL20R1/IL20R2</u>	<u>Gly4</u>	<u>264.35</u>
	IL22R1/IL20R2	none	25.26

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	IL22R1/IL20R2	IL-20	284.63
	IL22R1/IL20R2	IL-19	30.99
	IL22R1/IL20R2	IL-24	436.34
	IL22R1/IL20R2	Gly1	69.60
5	IL22R1/IL20R2	Gly2	294.24
	IL22R1/IL20R2	Gly3	31.87
	IL22R1/IL20R2	Gly4	30.66
	Ratio IL20R1/IL20R2 no ligand		1.01
	IL22R1/IL20R2	IL-20	1.36
10		IL-19	12.66
		IL-24	0.82
		Gly1	3.22
		Gly2	1.30
		Gly3	3.67
15		Gly4	8.62

Example 6 Polypeptide Expression in CHO Cells

The vector pC4 is one exemplary vector used for the expression of a polypeptide of interest in CHO cells. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. CHO cells or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (aha minus MEM, Invitrogen) supplemented with methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107, 1990; and M. J. Page and M. A. Sydenham, Biotechnology 9:64, 1991. Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are

obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains the LTR strong promoter of the Rous Sarcoma Virus (Cullen, *et al.*, Molec. Cell. Biol. 5:438, 1985) plus a fragment isolated from the enhancer of the immediate early gene of human CMV (Boshart, *et al.*, Cell 41:521, 1985).
5 Downstream of the promoter are restriction enzyme cleavage sites that allow insertion of the genes. Downstream of these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for expression, (*e.g.*, human b-actin promoter, SV40 early or late promoters, 10 or the LTR from other retroviruses). Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the polypeptide of interest in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547, 1992). For the polyadenylation of the mRNA, polyadenylation signals, (*e.g.*, from the human growth hormone or globin genes) can be used. Stable cell lines carrying 15 a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a gene expressing a selectable marker such as *gpt*, *G418* or *hygromycin*. It is advantageous to use more than one selectable marker in the beginning, *e.g.*, *G418* plus methotrexate.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for 20 transfection. Five micrograms of the expression plasmid containing the gene of interest is cotransfected with 0.5 μ g of the plasmid pSV2-neo using *e.g.*, lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the *neomycin resistance* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including *G418*. The cells are seeded in aha minus MEM supplemented with 1 μ g/ml *G418*. After 2 days, the 25 cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in aha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 μ g/ml *G418*. After about 10-14 days clonal colonies are independently trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of 30 methotrexate are then independently transferred to a new well of a 6-well plate containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100

- 200 mM methotrexate. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE, Western blot or by reverse phase HPLC analysis.

Example 7 in vitro Testing for Hematopoietic Modulators

5 a. Human Megakaryocyte Assay

Polypeptides can be assayed for their ability to stimulate development of human megakaryocytes from CD34+ progenitor cells. CD34+ selected cells are obtained from bone marrow as described (Hokom, M. *et al.*, *Molecular Biology of Haematopoiesis* 3:15, 1994) and incubated in Iscove's modified Dulbecco's medium (IMDM; GIBCO, Grand Island, NY) with 2 mM Glutamine, 2-mercaptoethanol (10^{-4} M), 1% bovine serum albumin, low density lipoprotein (40 µg/ml, Sigma); bovine pancreatic insulin (10 µg/ml), human transferrin (200 µg/ml), human recombinant thrombopoietin (50 ng/ml, R&D System); human recombinant stem cell factor (50 ng/ml, R&D Systems) and plus or minus isolated polypeptide of interest at various concentrations (typically about 200 ng/ml). CD34+ cells are plated at 3300 cells/ml final concentrations on 2 well chamber slides purchased from StemCell Technologies (Vancouver, Canada). Cells are incubated at 37°C for 12 days in humidified boxes in 5% CO₂ in air. The cells are then fixed directly to the culture wells with 1:3 methanol:acetone solution, and incubated with a monoclonal antibody, anti-GPIIb/IIIa, (StemCell Technologies). The immune reaction is developed with biotin-conjugated goat-anti-mouse Ig G followed by avidin-alkaline phosphatase conjugate, identified by pink color, and counted with an inverted-phase microscope at 100X magnification.

b. Liquid Bone Marrow Culture Assay

25 CD34⁺ human bone marrow cells (Poietics, Inc.) are plated in polypropylene V-bottomed 96 well plates at 10,000 cells/well with three wells/group. Stem Cell Factor (SCF) is used at 10 ng/ml, interleukin-3 (IL-3) is used at 0.1 ng/ml, erythropoietin (EPO) is used at 1 U/ml and the polypeptide of interest is used at various concentrations, (typically about 400 ng/ml). The following cytokine conditions are tested in IMDM/30% FBS + antibiotics: (1) SCF/IL-3/(± polypeptide of interest), (2) IL-3/EPO/(± polypeptide of interest); (3) SCF/IL-3/EPO/(± polypeptide of interest); (4) SCF/IL-3/macrophage colony stimulating factor; and (5) SCF/IL-3/EPO/TGFβ.

Sample 1, SCF + IL-3 is a negative control with minimal growth expected in the absence of an additional factor (e.g., IL-20). Sample 2, IL-3 + EPO is a negative control with minimal growth expected in the absence of an additional growth factor. Sample 3, SCF + IL-3 + EPO is expected to produce strong erythroid growth and/or differentiation in the absence of an additional factor and also demonstrates the amount of erythroid growth and/or differentiation in excess of that observed when SCF + IL-3 or IL-3 + EPO are used in the absence of the third factor (plus or minus IL-20). Sample 4, SCF + IL-3 + MCSF is used to demonstrate detectable monocytic growth and/or differentiation in comparison to using SCF + IL-3 in the absence of MCSF (plus or minus IL-20). Sample 5, SCF + IL-3 + EPO + TGF β is used to demonstrate modulation of erythroid growth and/or differentiation in comparison to using SCF + IL-3 + EPO in the absence of TGF β (plus or minus IL-20).

Cultures are incubated at 37°C, 5% CO₂, 95% humidity for 10 days with a breathable sealing membrane to prevent evaporation. Feeding occurs at days 4 and 7 by replacing 400 μ l of the medium with fresh medium. At day 10 the cells are transferred to V-bottomed plates and stained for CD14 (FITC) and CD36 (PE) cell surface antigens. The cells are centrifuged and incubated 15 minutes at 4°C with 50 μ g/ml human IgG (Sigma). Monocytes are CD14+, cells of the erythrocyte lineage are CD36+, cells that are negative for both CD14 and CD36 are termed "undefined" and may subsequently differentiate into monocytes or erythrocytes. Diagnostic antibodies, α CD14-FITC (Miltenyi Biotec) for monocytes and α CD36-PE (BD Pharmingen) for erythroid cells are added for an additional 15 minutes. After a wash (phosphate buffered saline, 0.1% bovine serum albumin), cells are transferred to 12 x 75 mm tubes in a final volume of 1 ml containing 0.1 ml FlowCount Fluorospheres (Coulter). Cells are then collected on a flow cytometer based on constant FlowCount Fluorosphere numbers, (e.g. 5000). Analysis of data is accomplished by determining the number of cells of each lineage that are present in each well which is calculated based on the known number of fluorospheres in the sample. Numbers of total cells, monocytic cells, erythroid cells, and undefined lineages (CD14- and CD36-minus) are determined. Data are subjected to statistical analysis.

c. Affect of Polypeptide on Number of CFU-GEMM

CD34⁺ cells are seeded into methylcellulose culture or Agar culture medium (Stem Cell Technologies) using standard procedures. Colony growth is stimulated with the following combinations of recombinant growth factors with and without polypeptide of interest (at about 200 ng/ml, or various test concentrations): (1) Epo (2 U/ml) plus SCF (50 ng/ml) and (2) Epo + SCF + IL-3 (10 ng/ml). All the commercially available cytokines are available from R&D Systems (Minneapolis, MN). After culture at 37°C for 2 weeks, the different types of colonies are counted from each dish under an inverted microscope. CFU-GEMM ultimately differentiate into red blood cells, granulocytes, monocytes, and platelets.

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Example 8 Additional *in vivo* Testing in Normal Mice for Hematopoietic Modulators

a. Assay for Recovery of Blood Cells after Bone Marrow Transplantation.

Bone marrow (BM) is harvested by gentle flushing the hind limbs of normal 8- to 10-week-old Balb C mice (purchased from Harlan Sprague Dawley, Indianapolis, IN) using RPMI medium containing 10% fetal calf serum. For some experiments, donor mice are pretreated with 5-fluorouracil (5-FU) at 150-mg/kg-body weight intraperitoneally (IP) 3 days before harvesting BM for infusion. After total body irradiation with about 10.8 Gy (¹³⁷Cs at 126cGy/min; split dose with a minimum of 3 hours between doses), 1 X 10⁶ bone marrow cells are injected intravenously (IV) into lethally irradiated mice. IL-20 (250 µg/kg body weight) is diluted in PBS and injected subcutaneously in 0.2-ml volume daily starting on the same day as irradiation and infusion of donor bone marrow cells. Control mice receive the same volume of PBS. Administration of polypeptides Gly1, Gly2, Gly3 or Gly4 occurs during days 0-17. Mice are weighed every 4 days during the post-transplantation period. Hematologic analysis of leukocyte cell counts and platelet counts are performed on orbit bleeds on a CDC Hemavet™ machine. Blood smears are stained with Wright-Giemsa using standard methods and examined at 100X for differentiation analysis. Hematocrits are performed by spinning capillary tubes for 5 minutes in a Model MB Micro-Capillary Centrifuge.

b. Assay for Recovery of Blood Cells after Combined Chemo-/Radiation Therapy
Eight- to ten-week old BDF1 mice (Harlan Sprague Dawley) are administered

carboplatin at 60-mg/kg body weights intraperitoneally (IP) 1 hour before sub-lethal irradiation (0.5 Gy total body irradiation for 20-22 mg mouse). A polypeptide of interest, e.g., Gly1, Gly2, Gly3 or Gly4 (with or without EPO or G-CSF) is injected subcutaneously in a 0.2 ml volume daily starting on the same day as irradiation. Negative control mice receive the same volume of PBS as the treated mice. Polypeptide administration lasts for 17 days. The mice are analyzed at various times post-radiation. Mice are weighed every 2 to 4 days during the post-radiation period. Hematologic analysis of leukocyte cell counts and platelet counts are performed on orbit bleeds on a CDC Hemavet™ machine. Blood smears are stained with Wright-Giemsa using standard methods and examine at 100X for differentiation analysis. Hematocrit measurements are performed by spinning capillary tubes for 5 minutes in a Model MB Micro-Capillary Centrifuge. Gly1, Gly2, Gly3 and/or Gly4 polypeptides may be useful in accelerating recovery of peripheral blood cell counts after chemotherapy and/or radiation therapy.

15 c. Anemia Treatment Assay

Various animal models of anemia and hematopoietic disorder are known in the art and generally accepted as being indicative of the anemic condition. For instance, the exhypoxic polycythemic mouse bioassay may be used to quantify the incorporation of ⁵⁹Fe (iron) into newly synthesized red blood cells as a measure of the increase in erythropoiesis in mice in response to an exogenously administered test sample. The assay, as described in WO/0024893, is a modification of the method of Cotes and Bangham (*Nature* 191:1065 (1961)).

The test agent(s) may be administered by any of several routes of administration (e.g. i.v., s.c., i.p., or by minipump or cannula) and suitable test animals include normal mice as well as transgenic mice similar to those described in Example 9. Controls for non-specific effects for these treatments are done using vehicle with or without the active agent of similar composition in the same type animal monitoring the same parameters.

Example 9 Transgenic animal development

30 Transgenic mice with the gene encoding Gly1, Gly2, Gly3 or Gly4 or other variants thereof, are generated using established techniques (Hogan, B. *et al.* (1986) *Manipulating the Mouse Embryo: A Laboratory Manual*. Cold Spring Harbor Laboratory,

NY as modified by Fox and Solter (Mol. Cell. Biol. 8: 5470, 1988). Briefly, a DNA fragment encompassing the human apolipoprotein E (hApoE) gene promoter-5'hApoE untranslated region-polypeptide of interest (Gly1-4)/FLAG-hepatic control region (HCR) fusion gene is purified by gel electrophoresis and glass bead extraction. The purified DNA fragment is microinjected into the male pronuclei of newly fertilized one-cell-stage embryos (zygotes) of the FVB/N strain. The embryos are cultured *in vitro* overnight to allow development to the two-cell-stage. Two-cell embryos are then transplanted into the oviducts of pseudopregnant ICR strain mice to allow development to term. To test for the presence of the transgene in the newborn mice, a small piece of toe is removed from each animal and digested with proteinase K to release the nucleic acids. A sample of the toe extract is subjected to PCR analysis using primers specific for the hApoE untranslated region to identify transgene-containing mice. Five founder transgenic mice are identified. Each of these founders is bred to produce F1 and F2 progeny. Transgenic mice are analyzed for the number CFU-GEMM in the spleen.

15 Example 10 Transgenic Mice Analysis

a. Hematopoietic Progenitor Cells

Mouse bone marrow cells and splenocytes are isolated from Gly1, Gly2, Gly3 and Gly4 (or variants thereof) transgenic mice and age-matched wild type mice. Then, 1 X 10⁵ mononuclear cells from the bone marrow or 1 X 10⁶ mononuclear cells from the spleen of each mouse are cultured in methylcellulose (Stem Cell Technologies) in the presence of 0.1 mM hemin using standard protocol known in the art. Colony growth is stimulated with the following combinations of recombinant growth factors: Medium A: Epo (1 U/ml, R&D System) plus SCF (50 ng/ml, R&D System) and PWM-SCM (conditioned medium 5%, Stem Cell Technologies) or Medium B: Epo 1 U/ml. After culturing the cells at 37°C for seven days, the different types of colonies are counted from each dish under an inverted microscope. Group mean and SD are calculated.

b. HCT Recovery in Transgenic Mice

Ten to twelve week-old transgenic mice and age matched, wild type mice are exposed to myelosuppressive therapy of 400 rads total body irradiation followed by a single intraperitoneal injection of 0.8 mg carboplatin as described by Kaushansky, *et al.* (J. Clin. Invest. 96:1883, 1996). For a comparison group, recombinant human Epo is

injected 20 IU daily s.c. for 12 days. Blood counts are performed on 50 μ l samples obtained by retro-orbital route, using a Hemavet 1500 hematology analyzer (CDC Technologies).

5 From the foregoing, it will be observed that numerous modifications and variations can be effected without departing from the true spirit and scope of the present invention. It is to be understood that no limitation with respect to the specific examples presented is intended or should be inferred. The disclosure is intended to cover by the appended claims modifications as fall within the scope of the claims.

What is claimed is:

1. A polypeptide comprising a glycosylation-modified IL-20 polypeptide which preferentially signals through one of the multiple IL-20 receptor complexes at a level at least 1.3 times the level it signals through another IL-20 receptor complex.
2. The polypeptide of claim 1, wherein the polypeptide preferentially signals through one of the multiple IL-20 receptor complexes at a level at least 2.0 times the level it signals through another IL-20 receptor complex.
3. The polypeptide of claim 1 or 2, wherein the receptor complex to which said polypeptide preferentially signals through comprises the IL20R1 and IL20R2 polypeptides (SEQ ID NOs: 22 and 3).
4. A glycosylation-modified IL-20 polypeptide, wherein the polypeptide comprises a sequence selected from the group consisting of:
 - a) amino acids 25 through 125 of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,
 - b) a sequence at least 95% identical to amino acids 25 through 125 of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,
 - c) a sequence at least 95% identical to amino acids 25 through 125 of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21,
 - d) amino acids 25 through 125 of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21.
 - e) amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20

and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,

f) a sequence at least 95% identical to amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs:

5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,

g) a sequence at least 95% identical to amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs:

5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21, and

h) amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21.

5. A glycosylation-modified IL-20 fusion polypeptide, wherein the polypeptide comprises a first portion and a second portion joined by a peptide bond and the first portion comprises a sequence selected from the group consisting of:

a) amino acids 25 through 125 of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,

b) amino acids 25 through 125 of a sequence at least 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,

c) amino acids 25 through 125 of a sequence at least 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21,

d) amino acids 25 through 125 of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21.

- 5 e) amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,
- 5 f) a sequence at least 95% homologous to amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21 in which an N-linked glycosylation site is located within three amino acids to either side of where it is in said sequence,
- 10 g) a sequence at least 95% homologous to amino acids 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21, and,
- 15 h) amino acid 25 through the carboxy terminus of a sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 14, 15, 16, 17, 18, 19, 20 and 21.
6. A polynucleotide comprising a sequence encoding a glycosylation-modified IL-20 polypeptide defined in claims 1, 2, 3, 4 or 5.
- 20 7. A polynucleotide comprising a sequence from the group consisting of:
- 25 a) nucleotides 75 through 375 of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13, in which the nucleotides encoding an N-linked glycosylation site is located within 9 nucleotides to either side of where it is in said sequence,
- 25 b) a sequence at least 95% identical to nucleotides 75 through 375 of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13, in which the nucleotides encoding an N-linked glycosylation site is located within 9 nucleotides to either side of where it is in said sequence,
- 30 c) a sequence at least 95% identical to nucleotides 75 through 375 of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13,

- 5 d) nucleotides 75 through 375 of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13,
- e) nucleotides 75 through the 3' end of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13, in which the nucleotides encoding an N-linked glycosylation site is located within 9 nucleotides to either side of where it is in said sequence,
- 10 f) a sequence at least 95% identical to nucleotides 75 through the 3' end of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13, in which the nucleotides encoding an N-linked glycosylation site is located within 9 nucleotides to either side of where it is in said sequence,
- g) a sequence at least 95% identical to nucleotides 75 through the 3' end of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13,
- 15 h) nucleotides 75 through the 3' end of a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 and 13,
- i) a sequence at least 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12 or 13 and,
- j) SEQ ID NOs: 10, 11, 12, or 13.

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8. A vector comprising a DNA sequence defined in claim 6 or 7.

9. The vector of claim 8, which is an expression vector.

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10. A vector according to claim 8, which is replicable in a prokaryotic and/or a eukaryotic host cell.

11. A vector according to claim 9, which is replicable in a eukaryotic cell.

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12. A vector according to claim 9, which is replicable in an insect cell.

13. A vector according to claim 9, which is replicable in a yeast cell.

14. A vector according to claim 11, which is replicable in a mammalian cell.
15. A vector according to claim 14, which is replicable in a Chinese Hamster Ovary cell.
- 5 16. A vector according to claim 14, which is replicable in a COS cell.
17. A host cell comprising the vector of claim 8.
- 10 18. A host cell according to claim 17, which is a prokaryotic cell.
19. A host cell according to claim 17, which is a eukaryotic cell.
20. A host cell according to claim 17, which is an insect cell.
- 15 21. A host cell according to claim 17, which is a yeast cell.
22. A host cell according to claim 20, which is a mammalian cell.
- 20 23. A host cell according to claim 22, which is a Chinese Hamster Ovary cell.
24. A host cell according to claim 22 which is a COS cell.
25. A process for preparing a glycosylation-modified IL-20 polypeptide of any one of
25 Claims 1 through 5 comprising culturing a suitable host cell comprising a vector
according to any one of Claim 8 through 16 under conditions promoting expression of the
polypeptide, and purifying said polypeptide.
26. A method of increasing the number of one or more type(s) of hematopoietic
30 progenitor cells in a mammal in need thereof comprising administering a therapeutically
effective amount of a glycosylation-modified IL-20 polypeptide described in claim 1, 4 or
5.

27. A method for treating or preventing a hematopoietic disorder in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.
28. A method of treating or preventing anemia, thrombocytopenia and/or neutropenia in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.
29. A pharmaceutical composition comprising a hematopoietic progenitor cell-stimulating amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5 and a pharmaceutically acceptable carrier, diluent or excipient.
30. A method for treating or preventing psoriasis in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.
31. A method for treating or preventing inflammation in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.
32. A method for treating or preventing cancer in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.
33. A method for treating or preventing a cardiovascular disorder in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical

composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.

5 34. A method for treating or preventing an immune system disorder in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5..

10 35. A method for treating or preventing obesity in a mammal comprising the administration to said mammal in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5.

15 36. A pharmaceutical composition comprising a psoriasis-relieving amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5 and a pharmaceutically acceptable carrier, diluent or excipient.

20 37. A pharmaceutical composition comprising an inflammation-decreasing amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5 and a pharmaceutically acceptable carrier, diluent or excipient.

25 38. A pharmaceutical composition comprising a CFU-GEMM stimulating amount of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5 and a pharmaceutically acceptable carrier, diluent or excipient.

39. Use of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1 through 5 for the preparation of a pharmaceutical composition to treat patients diagnosed with a hematopoietic disorder.

30 40. The use of Claim 38 wherein the hematopoietic disorder is anemia, thrombocytopenia or neutropenia.

41. The use of Claim 39 or Claim 40 wherein the composition is administered in an amount effective to stimulate hematopoietic progenitor cell differentiation.
42. Use of a glycosylation-modified IL-20 polypeptide described in any one of Claims 1
5 through 5 for the preparation of a pharmaceutical composition to treat patients with psoriasis, inflammation, cancer, cardiovascular disorder, or immune system disorder.

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Cys Val Ile Ala Thr Asn Leu Gln Glu Ile Arg Asn Gly Phe Ser Glu
 35 40 45

Ile Arg Gly Ser Val Gln Ala Lys Asp Gly Asn Ile Asn Xaa Xaa Xaa
 50 55 60

Ile Leu Arg Arg Thr Glu Ser Leu Gln Asp Thr Lys Pro Ala Asn Arg
 65 70 75 80

Cys Cys Leu Leu Arg His Leu Leu Arg Leu Tyr Leu Asp Arg Val Phe
 85 90 95

Lys Asn Tyr Gln Thr Pro Asp His Tyr Thr Leu Arg Lys Ile Ser Ser
 100 105 110

Leu Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp Leu Arg Leu Cys His
 115 120 125

Ala His Met Thr Cys His Cys Gly Glu Glu Ala Met Lys Lys Tyr Ser
 130 135 140

Gln Ile Leu Ser His Phe Glu Lys Leu Glu Pro Gln Ala Ala Val Val
 145 150 155 160

Lys Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln Trp Met Glu Glu Thr
 165 170 175

Glu

<210> 18

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<212> PRT

<213> Homo sapiens

X-15530.ST25.txt

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<223> X = Thr or Ser

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Leu Leu Trp Thr Pro Ser Thr Gly Leu Lys Thr Leu Asn Leu Gly Ser
 20 25 30

Cys Val Ile Ala Thr Asn Leu Gln Glu Ile Arg Asn Gly Phe Ser Glu
 35 40 45

Ile Arg Gly Ser Val Gln Ala Lys Asp Gly Asn Ile Asn Xaa Xaa Xaa
 50 55 60

Ile Leu Arg Arg Thr Glu Ser Leu Gln Asp Thr Lys Pro Ala Asn Arg
 65 70 75 80

Cys Cys Leu Leu Arg His Leu Leu Arg Leu Tyr Leu Asp Arg Val Phe
 85 90 95

x-15530.ST25.txt

Lys Asn Tyr Gln Thr Pro Asp His Tyr Thr Leu Arg Lys Ile Ser Ser
 100 105 110

Leu Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp Leu Arg Leu Cys His
 115 120 125

Ala His Met Thr Cys His Cys Gly Glu Glu Ala Met Asn Xaa Xaa Xaa
 130 135 140

Ser Gln Ile Leu Ser His Phe Glu Lys Leu Glu Pro Gln Ala Ala Val
 145 150 155 160

Val Lys Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln Trp Met Glu Glu
 165 170 175

Thr Glu

<210> 19

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<212> PRT

<213> Homo sapiens

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Met Lys Ala Ser Ser Leu Ala Phe Ser Leu Leu Ser Ala Ala Phe Tyr
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Leu Leu Trp Thr Pro Ser Thr Gly Leu Lys Thr Leu Asn Leu Gly Ser
 20 25 30

Cys Val Ile Ala Thr Asn Leu Gln Glu Ile Arg Asn Gly Phe Ser Glu
 35 40 45

Ile Arg Gly Ser Val Gln Ala Lys Asp Gly Asn Ile Asp Ile Arg Ile
 50 55 60

Leu Arg Arg Thr Glu Ser Leu Gln Asp Thr Lys Pro Ala Asn Arg Cys
 65 70 75 80

Cys Leu Leu Arg His Leu Leu Arg Leu Tyr Leu Asp Arg Val Phe Lys
 85 90 95

Asn Xaa Xaa Xaa Pro Asp His Tyr Thr Leu Arg Lys Ile Ser Ser Leu
 100 105 110

Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp Leu Arg Leu Cys His Ala
 115 120 125

His Met Thr Cys His Cys Gly Glu Glu Ala Met Asn Xaa Xaa Xaa Ser
 130 135 140

Gln Ile Leu Ser His Phe Glu Lys Leu Glu Pro Gln Ala Ala Val Val
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Lys Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln Trp Met Glu Glu Thr
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Glu

<210> 20

x-15530.ST25.txt

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Met Lys Ala Ser Ser Leu Ala Phe Ser Leu Leu Ser Ala Ala Phe Tyr
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Leu Leu Trp Thr Pro Ser Thr Gly Leu Lys Thr Leu Asn Leu Gly Ser
 20 25 30

Cys Val Ile Ala Thr Asn Leu Gln Glu Ile Arg Asn Gly Phe Ser Glu
 35 40 45

Ile Arg Gly Ser Val Gln Ala Lys Asp Gly Asn Ile Asn Xaa Xaa Xaa
 50 55 60

x-15530.ST25.txt

Ile Leu Arg Arg Thr Glu Ser Leu Gln Asp Thr Lys Pro Ala Asn Arg
 65 70 75 80

Cys Cys Leu Leu Arg His Leu Leu Arg Leu Tyr Leu Asp Arg Val Phe
 85 90 95

Lys Asn Xaa Xaa Xaa Pro Asp His Tyr Thr Leu Arg Lys Ile Ser Ser
 100 105 110

Leu Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp Leu Arg Leu Cys His
 115 120 125

Ala His Met Thr Cys His Cys Gly Glu Glu Ala Met Lys Lys Tyr Ser
 130 135 140

Gln Ile Leu Ser His Phe Glu Lys Leu Glu Pro Gln Ala Ala Val Val
 145 150 155 160

Lys Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln Trp Met Glu Glu Thr
 165 170 175

Glu

<210> 21

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Met Lys Ala Ser Ser Leu Ala Phe Ser Leu Leu Ser Ala Ala Phe Tyr
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Leu Leu Trp Thr Pro Ser Thr Gly Leu Lys Thr Leu Asn Leu Gly Ser
 20 25 30

Cys Val Ile Ala Thr Asn Leu Gln Glu Ile Arg Asn Gly Phe Ser Glu
 35 40 45

Ile Arg Gly Ser Val Gln Ala Lys Asp Gly Asn Ile Asn Xaa Xaa Xaa
 50 55 60

Ile Leu Arg Arg Thr Glu Ser Leu Gln Asp Thr Lys Pro Ala Asn Arg
 65 70 75 80

Cys Cys Leu Leu Arg His Leu Leu Arg Leu Tyr Leu Asp Arg Val Phe
 85 90 95

X-15530.ST25.txt

Lys Asn Xaa Xaa Xaa Pro Asp His Tyr Thr Leu Arg Lys Ile Ser Ser
 100 105 110
 Leu Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp Leu Arg Leu Cys His
 115 120 125
 Ala His Met Thr Cys His Cys Gly Glu Glu Ala Met Asn Xaa Xaa Xaa
 130 135 140
 Ser Gln Ile Leu Ser His Phe Glu Lys Leu Glu Pro Gln Ala Ala Val
 145 150 155 160
 Val Lys Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln Trp Met Glu Glu
 165 170 175
 Thr Glu

<210> 22
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<400> 22
 Met Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Pro Leu Leu Leu Leu
 1 5 10 15
 Leu Leu Ala Ala Pro Trp Gly Arg Ala Val Pro Cys Val Ser Gly Gly
 20 25 30
 Lys Pro Ala Asn Ile Thr Phe Leu Ser Ile Asn Met Lys Asn Val Leu
 35 40 45
 Gln Trp Thr Pro Pro Glu Gly Leu Gln Gly Val Lys Val Thr Tyr Thr
 50 55 60
 Val Gln Tyr Phe Ile Tyr Gly Gln Lys Lys Trp Leu Asn Lys Ser Glu
 65 70 75 80
 Cys Arg Asn Ile Asn Arg Thr Tyr Cys Asp Leu Ser Ala Glu Thr Ser
 85 90 95
 Asp Tyr Glu His Gln Tyr Tyr Ala Lys Val Lys Ala Ile Trp Gly Thr
 100 105 110

X-15530.ST25.txt

Lys Cys Ser Lys Trp Ala Glu Ser Gly Arg Phe Tyr Pro Phe Leu Glu
 115 120 125
 Thr Gln Ile Gly Pro Pro Glu Val Ala Leu Thr Thr Asp Glu Lys Ser
 130 135 140
 Ile Ser Val Val Leu Thr Ala Pro Glu Lys Trp Lys Arg Asn Pro Glu
 145 150 155 160
 Asp Val Ser Met Gln Gln Ile Tyr Ser Asn Leu Lys Tyr Asn Val Ser
 165 170 175
 Val Leu Asn Thr Lys Ser Asn Arg Thr Trp Ser Gln Cys Val Thr Asn
 180 185 190
 His Thr Leu Val Leu Thr Trp Leu Glu Pro Asn Thr Leu Tyr Cys Val
 195 200 205
 His Val Glu Ser Phe Val Pro Gly Pro Pro Arg Arg Ala Gln Pro Ser
 210 215 220
 Glu Lys Gln Cys Ala Arg Thr Leu Lys Asp Gln Ser Ser Glu Phe Lys
 225 230 235 240
 Ala Lys Ile Ile Phe Trp Tyr Val Ile Ser Ile Thr Val Phe Leu Phe
 245 250 255
 Ser Val Met Gly Tyr Ser Ile Tyr Arg Tyr Ile His Val Gly Lys Glu
 260 265 270
 Lys His Pro Ala Asn Leu Ile Leu Ile Tyr Gly Asn Glu Phe Asp Lys
 275 280 285
 Arg Phe Phe Val Pro Ala Glu Lys Ile Val Ile Asn Phe Ile Thr Leu
 290 295 300
 Asn Ile Ser Asp Asp Ser Lys Ile Ser His Gln Asp Met Ser Leu Leu
 305 310 315 320
 Gly Lys Ser Ser Asp Val Ser Ser Leu Asn Asp Pro Gln Pro Ser Gly
 325 330 335
 Asn Leu Arg Pro Pro Gln Glu Glu Glu Glu Val Lys His Leu Gly Tyr
 340 345 350
 Ala Ser His Leu Met Glu Ile Phe Cys Asp Ser Glu Glu Asn Thr Glu
 355 360 365

x-15530.ST25.txt

Gly Thr Ser Leu Thr Gln Gln Glu Ser Leu Ser Arg Thr Ile Pro Pro
 370 375 380
 Asp Lys Thr Val Ile Glu Tyr Glu Tyr Asp Val Arg Thr Thr Asp Ile
 385 390 395 400
 Cys Ala Gly Pro Glu Glu Gln Glu Leu Ser Leu Gln Glu Glu Val Ser
 405 410 415
 Thr Gln Gly Thr Leu Leu Glu Ser Gln Ala Ala Leu Ala Val Leu Gly
 420 425 430
 Pro Gln Thr Leu Gln Tyr Ser Tyr Thr Pro Gln Leu Gln Asp Leu Asp
 435 440 445
 Pro Leu Ala Gln Glu His Thr Asp Ser Glu Glu Gly Pro Glu Glu Glu
 450 455 460
 Pro Ser Thr Thr Leu Val Asp Trp Asp Pro Gln Thr Gly Arg Leu Cys
 465 470 475 480
 Ile Pro Ser Leu Ser Ser Phe Asp Gln Asp Ser Glu Gly Cys Glu Pro
 485 490 495
 Ser Glu Gly Asp Gly Leu Gly Glu Glu Gly Leu Leu Ser Arg Leu Tyr
 500 505 510
 Glu Glu Pro Ala Pro Asp Arg Pro Pro Gly Glu Asn Glu Thr Tyr Leu
 515 520 525
 Met Gln Phe Met Glu Glu Trp Gly Leu Tyr Val Gln Met Glu Asn
 530 535 540